PARASITOLOGY

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PARASITOLOGY

EDITED BY

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THE LIFE-HISTORY AND CHROMOSOME CYCLE OF *AGGREGATA EBERTHI* [PROTOZOA: SPOROZOA: COCCIDIA].

BY CLIFFORD DOBELL, F.R.S.

National Institute for Medical Research, London, N.W. 3.

(With Plates I–VI and 3 Text-figures.)

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I. INTRODUCTION

In the following pages I shall attempt to give an account of the chief results of a piece of work which I began sixteen years ago, and which I have continued—with numerous interruptions—ever since. Some of the facts here recorded have already been briefly summarized in two preliminary notes[1].

Originally the chief object of my work was to make a careful study of the development of the nuclei, and especially of the chromosomes, in one of the Coccidia. The classical researches of Siedlecki, Schaudinn, and others, revealed the life-cycle of these parasites in its general outline, but left much to be discovered in regard to the details: and it is not too much to say that in 1908, when I began my work, next to nothing was known about the chromosomes of the Coccidia, while not a little of what was then known about their nuclear development has since turned out to be inaccurate. I selected the form here described—*Aggregata eberthi*, a parasite of cuttlefish and crabs, in which it lives alternately—on account of its large size and frequent occurrence, and also because of its interesting life-history which was not fully elucidated at the time when I began my work.

As the work progressed, and I saw the interest which its results were likely to possess from a more general standpoint, I determined to publish all my observations in full detail. I intended to describe and illustrate every stage in the whole life-cycle of *A. eberthi*—every stage in every nuclear division, and every chromosome at every stage of development. I thought it desirable that such a complete description should be given for at least one member of the Coccidia. But this plan, though quite possible, and once apparently feasible, I have had to abandon: for it would require more time than I have at my command, and the cost of publishing such a monograph is now prohibitive. The present paper is therefore a compromise.

In the course of my studies I have received much assistance from various sources. I began the work, and collected most of the material on which it is based, at the Zoological Station at Naples in 1908. Here I occupied the Table of the British Association, with the aid of a grant from the Goldsmiths' Company. To both these bodies I desire to express my gratitude and thanks. I wish also to record my indebtedness to the former scientific staff of the Naples Station, but more especially to the late Dr Lo Bianco, who took great trouble to supply me with abundant fresh material and gave me invaluable help in its identification. I collected a little more material at Monaco in 1913, and I would here again thank the Oceanographic Museum for its hospitality, and my friend Dr Mieczysław Oxner for his kind help during my visit. In 1908 and 1909 I held the Balfour Studentship of Cambridge University, and with this assistance I was enabled to continue my researches at Cambridge. Afterwards I carried them much further at the Imperial College of Science (1910–

[1] See Dobell (1914) and Dobell and Jameson (1915). The present memoir was completed in July, 1924.

1915); and I have completed them—or more accurately, I have ended them for the present—at the National Institute for Medical Research, where the Council's enlightened concept of "Medical Research" has permitted me to pursue my zoological studies in freedom.

At Naples, in Cambridge, and in London, I have been helped in various ways by so many people that it is impossible, for lack of space, to do more than thank them here collectively. But I recall with gratitude, mingled with sorrow, the name of my dead Czechish friend Dr Emanuel Mencl of Prague—an admirable cytologist whose comradeship and counsel helped me over many a difficulty at Naples: and I must also particularly thank another friend, Dr A. Pringle Jameson, who, when working with me in London, kindly controlled many of my counts of chromosomes and checked my observations on numerous other points of cytological detail. I am deeply indebted to him for his expert help and criticism in this connexion.

Nor can I omit to mention the name of one whose aid alone made it possible for me to begin this work, and whose unfailing support in early days encouraged me to continue it. I refer to the late Prof. Adam Sedgwick, F.R.S., by whose death in 1913 I suffered an irreparable personal loss. If the following pages contain anything of scientific value, it is largely due to the help, inspiration, instruction, and not least of all to the friendship, of that great zoologist. I am only too conscious that my work is an inadequate return for all that he did for me—as inadequate, indeed, as these few words are to express my indebtedness and gratitude to him, and my lifelong veneration of his memory.

II. HISTORIC

Much has already been written about the development of our knowledge of the Aggregatidae, and an excellent summary of the subject—up to the time when it was written—has been published by Léger and Duboscq (1908). I shall therefore give merely an outline of the history of this matter here—noting a few omissions and errors in earlier accounts, and bringing the story up to date.

Aggregata eberthi is a common protozoal parasite of cuttlefish (*Sepia officinalis*) and crabs (Portunidae); and as it is, in certain stages of development, large enough to be visible to the naked eye, one might suppose that it would have attracted the notice of early workers. In spite of considerable research into the literature, however, I have been unable to find any evidence that it was seen by any observer before the middle of last century. Certainly the early microscopists who studied and described cuttlefish (Swammerdam, Redi, Needham, etc.) appear to have left no record, in their published writings, indicating that they ever noticed *Aggregata* in the course of their dissections.

It is generally believed that *A. eberthi* was discovered in *Sepia* by Lieberkühn in 1854[1], but this is incorrect. The real discoverer is, so far as I have been able to ascertain, unknown. Lieberkühn (1854), in a frequently cited

[1] Cf. Léger and Duboscq (1908), p. 47: "c'est Lieberkühn qui le premier (1854–55) vit, dans la Seiche, une psorospermie."

passage[1], does, indeed, mention that "gregarines...occur in Sepias": and it is now evident, from his brief remarks about them (only five-and-a-half lines), that he was referring to *A. eberthi*. There is thus no doubt that he saw this parasite. But he did not discover it: for in another work, written earlier but not published until later in the same year, he recorded a few further details which have been generally overlooked. In this paper he states[2] that an acquaintance—whom he does not name—gave him a piece of the gut (evidently the caecum) of a *Sepia* containing the parasites, together with drawings of them; and that after examining this material he was able to confirm this person's observations. It is thus clear that *A. eberthi* was discovered not by Lieberkühn but by his anonymous friend.

All that Lieberkühn himself (1854, 1854*a*) tells us about the parasites is that they are of variable size but more or less spherical or oval, and composed of finely granular protoplasm containing a remarkably distinct nucleus and bounded by a membrane. Some of them, he observes, encyst solitarily, and the cysts contain spores ("psorosperms" or "pseudonavicellae") which are roughly elliptical. His descriptions were very brief, but he gave a few figures[3]; and he considered the parasite to be a gregarine—a good determination for those days.

The next person to study *Aggregata* was Eberth (1862), who records that he found "encapsuled gregarines, with their psorosperms [= spores]" in "Octopus and Sepia vulgaris [= *Octopus vulgaris* and *Sepia officinalis*]" at Nice during June and July 1861. He figures and describes various stages, though without understanding them properly: but he notes that the "gregarine" has a large nucleus, with 1, 2, or 4 "nucleoli"; that it has an envelope; and that it breaks up into psorosperms (= spores) "without previous conjugation" (*i.e.* association in pairs). He saw the spores of more than one species, and also made out the sporozoites ("rolled-up threads") within them, and he first saw and figured the microgametes ("hairs"). On the whole, a good paper for its period; but perhaps hardly good enough to have justified Labbé (1896) in attaching Eberth's name to the *Aggregata* of *Sepia* for ever.

Apart from a slight notice by P. J. Van Beneden[4], the next work on *Aggregata* is that of Aimé Schneider (1875, 1875*a*, 1883), who added much new information. He first described the *Aggregata* of *Octopus*, and named it *Bene-*

[1] Lieberkühn (1854), Fortsetzung, p. 367.

[2] Lieberkühn (1854*a*), p. 9. As this work is not readily accessible to everybody I will quote the passage here in full: "Une personne de ma connaissance me céda quelques dessins de corps qu'il avait trouvés dans le ventricule (partie de l'estomac comparable au feuillet) de *Sepia officinalis*, et les accompagna d'un morceau de ce ventricule qu'il avait conservé dans de l'esprit-de-vin. En ramollissant ce dernier avec de la glycérine, étendue de beaucoup d'eau, j'étais en état de vérifier les dessins." This memoir is marked "couronné le 15 déc. 1854," and was published in the volume for 1854–55 (dated on title-page 1855). The exact date of publication I do not know; but that the paper was written earlier than that published in *Müller's Archiv* in 1854 is attested by Lieberkühn's own statement (1854, p. 17).

[3] Lieberkühn (1854*a*), Pl. VIII, figs. 9–12.

[4] Van Beneden (1875), pp. 145, 220: Text-figs. 34, 73 [oöcysts of *A. eberthi* containing spores].

denia octopiana (1875); but subsequently (1883) he studied the parasites of *Sepia* also (now called *A. eberthi*), and included both in the same species—whose name he changed, however, to *Klossia octopiana*. He saw many stages of development in the cephalopod hosts, and ultimately came to the correct conclusion that the parasites were not Gregarines but Coccidia.

Shortly afterwards Frenzel (1885) discovered the asexual stages of *A. eberthi* in a crab—*Portunus arcuatus*: but he did not know that his parasites were in any way connected with those described from cuttlefish, and he confused them with gregarines living in the same crab—putting them together in a "new species" for which he proposed the name *Aggregata portunidarum*[1].

Subsequently *A. eberthi* was studied again in *Sepia* by Mingazzini (1890, 1892, 1892*a*, 1893) and by Labbé (1895, 1896, 1899)—neither of whom can be said to have added much to our knowledge: but both regarded the parasite as a member of the Coccidia, and Labbé (1896), after discussing the nomenclature, proposed the name *Klossia eberthi* for it—though leaving (1899) the schizogonous forms, of course, where Frenzel had put them. In his large systematic work on the Sporozoa, Labbé (1899, p. 54) by an unfortunate error attributed the first observations on *A. eberthi* to Van Beneden[2].

By far the best work done on *A. eberthi* during this period is that of Siedlecki (1898, 1898*a*). Considering the time when it was written (the life-cycle of the Coccidia had then only just been elucidated by Schaudinn and Siedlecki), Siedlecki's memoir on *Aggregata* must be regarded as very good indeed. It contains mistakes, of course, which subsequent workers have not failed to point out: but it also contains a large number of accurate and novel observations. In this work Siedlecki (1898) described correctly, for the first time, the whole sexual cycle and sporogony of *A. eberthi* in *Sepia*: he described the development of the microgametes and macrogametes, and the process of fertilization: and on the basis of his observations he correctly referred the organism to the Coccidia. I shall have to discuss, and sometimes to correct and amplify, various details of Siedlecki's work in later pages: nevertheless, I would say here that it is, in my opinion, a work of such general excellence that it constitutes one of the classics of protozoology.

Aggregata (*i.e.* the sexual form in Cephalopoda) was discussed—chiefly from the standpoint of nomenclature and systematics—by various workers in the next few years. It will suffice to mention here the works of Blanchard (1900), Doflein (1901), Lühe (1902, 1903), Minchin (1903), and Jacquemet

[1] Frenzel's "*Aggregata portunidarum*" comprised the schizogonous forms of *A. eberthi* (in *P. arcuatus*) and of the *Aggregata* (? sp.) found in *Carcinus maenas*, together with the gregarine *Porospora portunidarum* (Frenzel) Léger and Duboscq, 1911 (= *Frenzelina portunidarum* (Frenzel) Léger and Duboscq, 1907*a*, 1908). But recently Léger and Duboscq (1913) have stated that *Porospora* is itself the schizogonous form of a sporozoon which undergoes its sporogony in the cockle (*Cardium edule*), in which host it had previously been described as a coccidium, under the name of *Nematopsis*. The constitution of "*Aggregata portunidarum*" is, even now, not completely elucidated. Cf. further Léger and Duboscq (1909, 1911*a*, 1917).

[2] Labbé's mistake has since been copied by Jacquemet (1903), who, in discussing the nomenclature, evidently omitted to verify his references.

(1903). Each of these writers introduced a new name, or new combination of names, for *A. eberthi*; but I need not discuss these here[1]. During the same period the asexual stages were also seen and studied in various crustacea by Léger (1901), Léger and Duboscq (1903), and G. Smith (1905).

The first real advance in our knowledge of *A. eberthi*, after the work of Siedlecki, was made by Léger and Duboscq (1906, 1906*a*, 1907, 1908), who showed by admirable experiments and observations that Frenzel's "gregarine" from *Portunus* and the "coccidium" of *Sepia* are in reality the asexual and sexual forms respectively of a single species of sporozoon. Léger and Duboscq (1908) gave an excellent account of the asexual cycle, adding many new details to those previously known, and discussed the systematics and nomenclature once more—in the light of their new discoveries, which had made a complete revision necessary. They finally concluded that the parasite of *Portunus* and *Sepia* is a Schizogregarine, whose proper name is *Aggregata eberthi* (Labbé). Details in the work of Léger and Duboscq will be discussed later, and it will be sufficient to emphasize here that their memoir of 1908 forms a landmark in the history of this subject.

In assigning *Aggregata* to the Gregarinida, and not to the Coccidia, Léger and Duboscq were obviously influenced by the work of Moroff, published at about the same time. In a series of preliminary papers, Moroff (1906, 1906*a*, 1907[2]), after restudying the species of *Aggregata* occurring in *Octopus* and *Sepia*, declared that Siedlecki's account of the development was wrong; and that fertilization does not occur at the stage at which he described it but immediately before spore-formation—Siedlecki's "sporoblasts" being really macrogametes. This being so, it followed that the organism was not a coccidian but a gregarine; and as it underwent schizogony in its alternative host—a crab —it followed that its proper systematic position was amongst the Schizogregarines. Moroff's whole case obviously depended upon his ability to prove that Siedlecki was mistaken, and that fertilization really occurs in the manner characteristic of gregarines: but although he expressed himself confidently[3] in his earlier notes, it became evident when he published his full account (Moroff, 1908) that he was unable to adduce any good evidence in support of his assertions. He brought forward no proof that Siedlecki's interpretation was incorrect, and nothing but doubtful evidence and hypotheses in support of his own: so that one must agree with Caullery[4] that, far from having proved his case, "on ne peut nier qu'à cet égard on n'éprouve une déception véritable" in his final publication.

Moroff's memoir (1908) is the biggest work that has hitherto been published on *Aggregata*—notwithstanding that more than 60 of its 224 pages are occupied

[1] *Vide* p. 110, *infra*.

[2] See also Moroff (1907*a*), pp. 33–4.

[3] *E.g.* "Siedlecki...a pris...la formation des macrogamètes pour un stade de sporulation, et en conséquence méconnu complètement le véritable processus sexuel." "En résumé, les prétendues Coccidies des Céphalopodes sont en réalité des Grégarines." "Ce sont des Grégarines monocystidées," etc. (Moroff, 1906, p. 654).

[4] M. Caullery (1908), review of Moroff (1908) in *Bull. Inst. Pasteur*, VI. 723.

by cytological generalities and speculations. It deals almost entirely, however, with the cycle in the cephalopod host, and most of his observations were made upon the parasites of *Octopus*. In this host and in *Sepia* he distinguished no less than 17 different species, which, with four others which he recognized (from crabs), brought the total number of species of *Aggregata* up to 21. There can now be no doubt that many of these species are invalid. Various other details in Moroff's work will be noted later: and I will merely add here that, though this work was published ten years after that of Siedlecki, whose observations it professes to correct, the Bulgarian zoologist's views and descriptions are, for the most part, less acceptable and accurate than those of his Polish precursor.

In 1908, when I began to study *Aggregata*, it was uncertain whether these parasites ought to be classified as Coccidia or Gregarines, though most zoologists regarded them as the latter—as the result of the work of Léger and Duboscq and of Moroff. It was clear that a complete and accurate study of the sexual cycle ought to be made, in order to decide whether Siedlecki's interpretation or Moroff's was correct; and it also seemed desirable that someone should repeat the experiments of Léger and Duboscq. I attempted to carry out the whole of this programme, but did not reach any definite conclusions for about five years[1]. At the end of 1913, however, I had collected sufficient evidence to prove that the work of Siedlecki and of Léger and Duboscq was essentially correct, while Moroff's interpretation of the life-cycle was unquestionably wrong. I therefore published a brief summary of my conclusions at that time (Dobell, 1914).

A few months later, a paper announcing a similar conclusion—namely, that *Aggregata* is a coccidian, and not a gregarine—was published by Pixell-Goodrich (1914)[2]. I think, however, that attentive study of her paper will convince anybody acquainted with the situation that it contains less evidence in support of such a conclusion than is to be found in the earlier work of Siedlecki (1898).

After I had succeeded in working out the chromosome cycle in detail, I published a second preliminary note (Dobell and Jameson, 1915), in which I recorded my findings very briefly. I stated that the chromosomes of *A. eberthi* are six in number at every nuclear division in the life-cycle except one—the reduction division, which takes place immediately after fertilization: that at this division they number 12, and are once more halved to six: and that no reduction occurs during gametogenesis in either the males or the females. The chromosome cycle is thus very remarkable; for all individuals—whether male, female, or asexual—contain the same number of chromosomes, and this number is, moreover, the haploid number. In the present paper I hope to be

[1] I should note, however, that from the very beginning of my studies I was sceptical of Moroff's observations, and impressed by the accuracy of Siedlecki's work. This is shown by a reference which I made to the subject in a paper written in the summer of 1908 (Dobell, 1909*a*, p. 295).

[2] This paper was published in April, 1914, while my preliminary note appeared in January of the same year.

able to furnish proofs of these and other statements made in my preliminary notes.

In the ordinary course of events I should have been able to publish a full account of my work on *A. eberthi* some years ago: but owing to the War my activities were directed into other channels and full publication has therefore been unavoidably delayed.

Since the appearance of my preliminary communications my conclusions have been accepted by several other workers, but no other original work on *Aggregata* has, to my knowledge, been published—with the exception of two very recent notes by Joyet-Lavergne (1924, 1924*a*), who has studied the "Golgi apparatus" in *A. eberthi*. I shall therefore say nothing more about previous work here, but will notice various details in the memoirs just noted—and in others—at relevant points in the ensuing pages.

III. MATERIAL AND METHODS

Most of my material, which was obtained from cephalopods and crabs, was collected at Naples in the months of March, April, May, and June, 1908. During this period I dissected and examined 310 cephalopods, belonging to 14 different species, and 50 crabs, of 4 different species, in order to determine whether they harboured *Aggregata*.

The results of all my examinations of Cephalopoda at Naples are summarized in Table I. In the nomenclature of the various species I have followed the well-known monograph of Jatta (1896), though I have ventured to substitute the more usual names Sepiolidae, Sepiidae, and Loliginidae, respectively, for his three families Sepiolini, Sepiarii, and Loliginei[1].

Table I.

CEPHALOPODA	No. examined	No. infected with *Aggregata*
DECAPODA.		
Fam. Ommatostrephidae		
1. *Illex coindetii* (Verany) Steenstrup ...	9	0
Fam. Sepiolidae		
2. *Sepiola rondeletii* (Gesner) Leach	55	0
3. *Rossia macrosoma* (Delle Chiaje) D'Orbigny	7	0
Fam. Sepiidae		
4. *Sepia officinalis* L.	73	73
5. *S. orbignyana* Férussac	8	0
6. *S. elegans* D'Orbigny	82	0
Fam. Loliginidae		
7. *Loligo vulgaris* Lamarck	13	0
8. *L. marmorae* Verany	16	0
OCTOPODA.		
Fam. Argonautidae		
9. *Ocythoe tuberculata* (Rafinesque) Steenstrup	3	0
Fam. Octopodidae		
10. *Octopus vulgaris* Lamarck	8	8
11. *O. macropus* Risso	2	0
12. *O. defilippi* Verany	7	0
13. *Eledone moschata* Lamarck	25	0
14. *E. aldrovandi* Rafinesque	2	0

[1] I have also amended "Ommastrephidae" and "Octopidae" to Ommatostrephidae and Octopodidae, for obvious reasons. The family names given in the Table are therefore identical with those in Sedgwick's *Text-Book of Zoology*, I. 1898.

From this Table it will be apparent that only two species of cephalopod were found to harbour *Aggregata*—namely, *Sepia officinalis* and *Octopus vulgaris*: but every individual of these two species was infected, and as a rule heavily infected[1].

The method of examination was as follows: The cephalopod was killed, and its alimentary canal dissected out and opened. The intestine, caecum, and stomach were then particularly examined with the aid of a hand-lens. An infection with *Aggregata* can usually be readily detected by these means; but if none were thus discoverable, samples of the lining of the gut were scraped off with a scalpel and examined under the microscope. If, after this procedure, no parasites were found, the cephalopod was recorded as "negative"; and only on rare occasions were pieces of the gut-wall of such individuals fixed and sectioned—the result, when this was done, being invariably a confirmation of the original negative diagnosis. On several occasions, however, the result of the microscopic examination was doubtful, as small whitish bodies were found which could not be definitely identified. These were fixed and sectioned, and in every case proved to be encysted cestodes[2]. While, therefore, I am certain that every individual recorded as "infected" was really infected with *Aggregata*, I am unable to guarantee that every "negative" host was not infected. All I can say with confidence is that all the "uninfected" individuals in my list were either uninfected or had an infection so small as to escape detection by the methods employed.

The individuals examined, of each species, were as a rule of various ages and both sexes: and in the infected forms the incidence was the same (100 per cent.) in both sexes and at all ages. I devoted my attention especially—after a preliminary survey—to *Sepia officinalis*, and this accounts for the large number of individuals of this species in the list. Some other species—*e.g.*, *Eledone moschata*—which were equally easily obtainable, were not studied further when I found that they were usually free from parasites. In the case of *Ocythoe tuberculata*, however, and some of the rarer cephalopods, the small numbers examined are due to the circumstance that more specimens were unobtainable. *S. officinalis* was plentiful, and was usually brought in alive and in good condition, and only fresh living individuals were used for my experiments. It should be noted, however, that some of the rarer species were dead when brought to the laboratory by the fishermen, and were therefore examined after death; but it is unlikely that this has influenced my findings for these

[1] Mingazzini (1892) says he found almost every *Sepia* infected at Naples, but *Octopus* less often. The species studied are not recorded, and he gives no indication of the number of individuals examined. It is also mentioned in this paper that *Rossia*, *Sepiola*, and *Argonauta* were found to be uninfected. Cf. also footnote on p. 10, *infra*.

[2] Apparently "*Scolex polymorphus*" (=metacestode of *Calliobothrium filicolle*); see Monticelli (1888). But I am not prepared to say that the encysted cestodes found were all of one species. I found these organisms—free, or encysted in the gut wall—in *Sepia officinalis*, *Eledone moschata*, *E. aldrovandi*, *Octopus vulgaris*, *O. macropus*, *Loligo vulgaris*, and *Ocythoe tuberculata*. Whether the "species of Trematode" observed by Moroff (1908, p. 138) in "*Loligo*, *Eledone*, and *Octocotyle*[?]" was a similar parasite I am unable to determine, though this seems not unlikely.

species, as *Aggregata* is easily recognizable—though quite unsuitable for cytological study—long after the death of its host.

Additional material which I collected at Monaco in September and October, 1913, was less satisfactory. Here I had difficulty in obtaining *S. officinalis* in large numbers and in good condition. Consequently I examined carefully only 20 individuals; and of these only 8 were found to be infected with *Aggregata*. It should be noted, further, that the uninfected specimens were mostly very small as compared with my Naples sepias, and two of them were dead when examined. Whether *S. officinalis* is normally more frequently infected at Naples than at Monaco, at all seasons, I am unable to state; but the difference observed—100 per cent. infected at Naples, 40 per cent. at Monaco—seems noteworthy[1].

At Naples I had no difficulty in keeping *S. officinalis* alive and well in the laboratory tanks for as long a period as I desired—sometimes for three or four weeks. The animals remained healthy and lively, and appeared to suffer in no way from their confinement. The females even laid their eggs—usually round the outflow pipe of the tank, which was provided with a constant stream of running water—and the larger individuals very soon became sufficiently tame to allow themselves to be handled and even removed from the water without discharging their ink-sacs or displaying the other signs of fear (colour changes) so characteristic of these cephalopods. But at Monaco I was unable to keep any sepias alive for more than a day or two at most, and I was therefore unable to carry out some of the experiments which I had planned.

As the work of Léger and Duboscq—then (1908) only just published—had shown that the *Aggregata* of *Sepia* undergoes its schizogonic development in crabs, it was to these crustacea that I devoted most of my attention when at Naples. The results of my experiments will be described in a later section, and I shall here merely record my general findings.

From the previously published observations it appeared to me probable that the intermediate host of *A. eberthi* at Naples would prove to be *Portunus depurator* or a species of *Inachus*. I soon found that several species of wild crabs were infected with *Aggregata*, and feeding experiments soon showed me that the parasites in *P. depurator* were really developmental forms of those in *Sepia*—in agreement with Léger and Duboscq's final conclusions. The only other *Portunus* that I was able to study was *P. corrugatus*, which I also found to be naturally infected with an *Aggregata*, though not so regularly

[1] Moroff's observations, so far as they go, seem to agree with mine in so far as the distribution of *Aggregata* in Mediterranean cephalopods is concerned: for he says (Moroff (1908), p. 138) that he found the parasites in *Octopus* and *Sepia* only, though unfortunately he omits to state what species of these genera he studied. (I presume that when he says "*Sepia*" he always means *S. officinalis*, whilst by "*Octopus*" he invariably means *O. vulgaris*.) He also records, however, that the only other cephalopods which he examined were "*Loligo, Eledone* and *Octocotyle*[?]"—no specific names being given, and no indication of the number of individuals examined. Pixell-Goodrich (1914) says she found all individuals of *S. officinalis* infected with *Aggregata* at Plymouth, the Solent, and Banyuls; and that at the last place *S. elegans* and *Eledone* and *Loligo* (no species stated) were not infected. The numbers of individuals examined are not recorded.

as *P. depurator*. I therefore concentrated my attention chiefly on the latter species, and performed few experiments with other crabs.

In Table II are summarized all the results—as regards infection with *Aggregata*—recorded finally from my examination of 50 wild crabs[1].

Table II.

CRABS	No. examined	No. infected	Percent. infected
1. *Portunus depurator* L.	28	21	75
2. *Portunus corrugatus* Pennant ...	5	2	40
3. *Carcinus maenas* Pennant ...	5	0	0
4. *Inachus mauretanicus* Lucas ...	12	1	8·3

As will be seen later, the *Aggregata* found in *Portunus depurator* in nature (which is probably identical with that found in *P. corrugatus*) is *A. eberthi*—the species which develops sexually in *Sepia officinalis*. The *Aggregata* found in *Inachus mauretanicus*, however, appears to be different. It is probably the same as that described from the same host, and also from *I. dorsettensis*, by Geoffrey Smith (1905), who named it *A. inachi*. With Léger and Duboscq I regard it as probably, in reality, the asexual form of one of the species of *Aggregata* which undergo their sexual cycle in *Octopus*.

The method adopted in examining these wild crabs was similar to that just described in the case of the cuttlefish, and the results are therefore open to the same objections. The number of infected specimens recorded is the minimal number certainly infected, while among those called "uninfected" some may have harboured parasites in such small numbers that they were not detected. Material from these naturally infected crabs was preserved, in almost every case, for cytological study; but the crabs of this series were not used for experimental purposes. Like the sepias, they lived very well in the laboratory tanks at Naples—my only trouble being to prevent them from occasionally climbing out of the water and escaping.

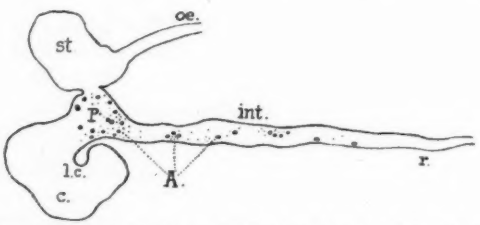
After I had determined that *Portunus depurator* was commonly infected with *A. eberthi*, and would best serve the purpose of my studies, I made no attempt to examine a large series of other species of crustacea in order to discover whether they also might harbour similar parasites. My results, therefore, add little to what is already known—chiefly from the work of Léger and Duboscq (1908)—concerning the occurrence of Aggregatidae in the Crustacea generally.

I may add here that neither in cephalopods nor in crabs did I meet with any sporozoan parasites except *Aggregata*. This is not surprising in the case of the cephalopods, since no other intestinal sporozoa have been reported from

[1] The names of the crabs in this Table are given on the authority of Dr W. T. Calman, F.R.S., who kindly assisted me in the matter of their nomenclature. I must point out that the species of *Inachus* here called *mauretanicus* is the same as that called *I. scorpio* by Léger and Duboscq (1908) and by Smith (1905); and it was by this name that it was known to me and other workers at Naples when I was there. For a brief systematic account of the Mediterranean species of *Portunus* (9 spp. recognized) see Parisi (1915).

these animals: but the absence of intestinal gregarines from all my crabs appears noteworthy, since, to judge from previous accounts, such parasites are by no means uncommon in the Decapod Crustacea of the Mediterranean. Most of the Cephalopoda which I examined contained Dicyemids in their kidneys, and some of them harboured Ciliates in the kidney or liver[1].

Aggregata eberthi lives in the submucous connective tissue of the gut of *Sepia officinalis*, only the very earliest stages in development being found in the epithelium of the mucous membrane[2]. The parasites occur in the whole of the intestine from its juncture with the stomach to within a short distance from the anus (see Text-fig. 1). I have never found any in the oesophagus (*oe.*) or stomach proper (*st.*); but the initial part of the intestine—which is dilated into a sort of pouch (*p.*)—and the caecum (*c.*) are generally well infected. The caecum, which is more or less spirally twisted, is anatomically a part of the intestine, though it is often described as an appendix of the



Text-fig. 1. Outline drawing of gut of a small *Sepia officinalis*—dissected out. Natural size.

stomach. In the living animal stomach and caecum lie closely apposed, but when the alimentary canal is dissected out it can easily be seen that the caecum is a diverticulum of the intestine, and not of the stomach (see Text-fig. 1). The mucous membrane of the lesser curvature of the caecum (*l.c.*) is thrown into a series of deep transverse folds[3], and these are usually densely packed with parasites—the rest of the organ being often but slightly infected. The long, straight intestine (*int.*) contains parasites which are generally most numerous in the region of the pouch (*p.*), and generally become fewer and more scattered towards its anal extremity or rectum (*r.*).

As a rule an infection with *Aggregata* can be detected by naked-eye examination alone—the parasites appearing as minute white dots (*A.*) in the gut wall. These white dots, which may attain a size of about 1 mm. in diameter, are oocysts packed with spores. The larger of them are formed by

[1] The Ciliata which were found in the series of cephalopods given in Table I have already been described in an earlier paper (Dobell, 1909).

[2] Mingazzini's statement (1890, p. 152) that the parasites can also live "free in the intestinal cavity" has been confirmed by no other observer and is certainly incorrect.

[3] The epithelial cells at the summits of these folds appear to be specially concerned in the absorption of fat: for in suitably stained preparations they can usually be seen to be packed with fat droplets, which are absent from the rest of the caecal epithelium. I have not found this noted elsewhere.

the coalescence of several smaller ones, and they attain their maximum size in the intestine (*int.*) and its initial dilatation (*p.*), where they are consequently most conspicuous. But the stages preceding spore-formation are not usually visible to the naked eye, and the number of white dots seen on casual examination is therefore not a trustworthy index of the degree of infestation of the gut as a whole.

I examined all stages in the development of *A. eberthi* in the living state, and also in fixed and stained smear preparations and in sections. For studying the finer details of structure, sections are indispensable, and most of my results are based upon such preparations. As I had abundant material at my disposal, I fixed and stained it in a great many different ways; but I shall here note only the more important points which have emerged from the results finally obtained in studying this material.

In the first place, I would lay great stress upon a point which is of the utmost importance for anybody who wishes to make a cytological study of *Aggregata*. It is that only material which is perfectly fresh has any value—if one desires to study the normal development. This is so obvious a truism that it would hardly seem worth mentioning, were it not that the majority of the mistakes of earlier workers seem to me clearly referable to inattention to this source of error—coupled with faulty technique.

Material from a dead cuttlefish—even if it has only just died—is worthless for cytological purposes, for most of the parasites in such an animal will be found in a more or less abnormal and degenerate condition. (This applies not only to *Aggregata* but also to the parasitic ciliates—*Chromidina* and *Opalinopsis*—and Dicyemidae.) In an almost incredibly short time—a matter of minutes only—after the death of their host, the parasites also begin to die. In sections (or smears) of material fixed only 5 minutes or so after the host (*Sepia*) has been killed, abnormal nuclear figures are usually to be seen—all too plentifully, as a rule—in the parasites (*A. eberthi*); and after about 10 minutes, there are usually more abnormal than normal stages. Only fully formed spores remain unchanged for any length of time, and a *Sepia* which has been dead for more than a few minutes is therefore worthless for the cytological study of any stages but these.

In order to obtain perfectly fresh material for fixation I finally used the following method, which alone gave really satisfactory results[1]. Selecting a sepia which is alive and vigorous, I remove it from the water and wrap it in a cloth (to soak up superfluous water and enable me to grip the animal firmly[2]). With stout bone-forceps I then cut through the back of the skull—almost severing the head—and then pin the animal down, belly upwards, in a dry dissecting dish. (The arms must be transfixed with strong pins, as the

[1] Siedlecki (1898), it may be noted, dissected his cuttlefish alive. Pixell-Goodrich (1914) recommends killing by strangulation. The first method is good—cytologically—but brutal: the second I have not tried.

[2] This is very necessary, as the animal is slippery and if improperly held can inflict a severe bite with its horny jaws—as I know only too well from painful experience.

animal generally continues to struggle violently.) A few cuts with a sharp scalpel suffice to lay bare the alimentary canal, and two cuts with the scissors —one through the oesophagus, the other through the extreme hind end of the intestine—enable it to be removed intact from the body (cf. Text-fig. 1). I then slit open the intestine and caecum with the scissors, and cut out—with the same instrument—parasitized portions of suitable size, and drop them immediately into the previously warmed fixing fluid in a test tube. It is most important to make certain that the parasites do not come in contact with sea-water or the juices from the intestine at any stage in this procedure[1]. I therefore not only wipe the sepia carefully with a cloth before I open its mantle chamber, but I remove all liquid from this also (with the cloth or blotting paper) before I cut into the body cavity: and when I have slit open the gut, I drain off its contents and carefully blot its inner surface before I cut out bits of its wall for fixation. Of course, one must also be careful not to puncture the ink-sac, or otherwise cause the discharge of its contents. The blood of the cuttlefish, however, does not appear to injure the parasites, and I usually mix this with scrapings of the intestinal wall for making smears and for examining the organisms alive.

Every step in the foregoing procedure should be effected as rapidly as possible. Delay at any stage—even for only a few minutes—will probably ruin the parasites for cytological purposes. It should be noted that the appearance of the organisms in the fresh state affords very little guidance for determining whether they are fit for preservation or not. I preserved a very large amount of material from recently killed cuttlefish because it appeared quite fresh and good under a low magnification—but only to find, when I sectioned it afterwards, that it was almost worthless for studying the nuclear changes. Only material fixed immediately after killing the host—while its tissues are still living—has any value for cytological study.

I fixed my material in many different ways. The best fixation of smears was obtained with Schaudinn's fluid, Bouin's fluid, and Flemming's (strong) fluid—the last being the best for small parasites, if the smears are thin and the fixative is not allowed to act for more than ten minutes. Blocks of tissue—for sections—cannot, however, be satisfactorily fixed in this mixture. I am aware that this is contrary to the statements of some other workers, but it has been my experience consistently. Flemming's (and also Hermann's) fluid often fixes the tissues of the host beautifully: but it will not penetrate sufficiently rapidly to fix a mass of closely packed parasites properly. Even isolated individuals, in smear preparations, if they happen to be large usually display obvious signs of bad fixation. Many of the appearances described and figured as normal stages by Moroff (1908) are, I believe, artifacts, for which his reliance upon Flemming's fluid was responsible. Such appearances, at all

[1] Pixell-Goodrich (1914) made her preparations by teasing up the gut wall in sea-water or intestinal fluid. How any cytologist can advocate such a method after once trying it, I am unable to understand.

events, are certainly not normal, and are not usually seen when other fixatives are used. Schaudinn's fluid did not always give satisfactory results with blocks of tissue. Bouin's fluid was usually better, as also was picro-acetic acid (3 parts saturated aqueous solution of picric acid, 1 part glacial acetic acid) which often fixes even very large parasites remarkably well. But the best fixation of all, for bits of gut wall containing the parasites, was obtained with a mixture consisting of mercuric chloride (3 parts of a saturated aqueous solution) and formalin (1 part of the strong solution containing 40 per cent. formaldehyde). This fixative gave very uniform results, with no visible shrinkage, swelling, or vacuolation of the parasites or their nuclei, and excellent fixation of the tissues of the host. Small bits of parasitized tissue were dropped into this mixture—usually warmed previously—and left in it, cold, for 24 hours, during which time the fluid was renewed at least once. They were then transferred directly to 70 per cent. alcohol and hardened in this fluid—changed several times to wash out excess of sublimate—before being sectioned. Acetic acid should not be added to this sublimate-formol mixture, and the tissues, after fixation, should always be thoroughly hardened in alcohol—as just noted—before they are imbedded. Other sublimate mixtures gave far less satisfactory results, and formalin alone I found to be a poor fixative for *Aggregata*. The same may be said of most of the mixtures recommended by previous writers for fixing this parasite. I have tried all of them, but am satisfied with none. Corrosive sublimate in sea-water, as recommended by Siedlecki (1898), was, in my hands, one of the worst.

For fixing smears of crabs' intestines containing parasites, I used chiefly Schaudinn's and Bouin's fluids, which both gave satisfactory results. I used these mixtures also to fix the guts of crabs intended for sectioning—dropping the whole midgut into the warmed fixative. Bouin's fluid proved to be the better of the two for this purpose. After fixation (usually for 24 hours) the guts were transferred to 70 per cent. alcohol and thoroughly hardened in this before being imbedded. Although I generally treated infested crabs' guts in this manner, I sometimes detached the coelomic cysts with a needle—when they were present in clusters, loosely adherent to the gut wall—and fixed and sectioned them separately from the rest of the intestine. Such clusters, being small, are rapidly penetrated by the fixative, and furnish very good preparations of certain stages. It must be noted that precautions similar to those mentioned in the case of the cuttlefish must be taken to prevent sea-water from coming in contact with the parasites in the tissues of crabs, for even a small quantity of sea-water ruins them for cytological study. Moreover, the absolute necessity of obtaining perfectly fresh material must be emphasized again here. Nothing worth having can be got out of a dead crab.

Most of my material for sectioning was imbedded in paraffin in the usual ways, but I also used, at times, the celloidin-paraffin double-imbedding process with good results. For studying the nuclei and chromosomes, perfect serial sections are, of course, absolutely necessary.

Although I cut many sections of medium thickness (5μ–10μ), I also, for special purposes, cut very thin sections (1μ, 2μ, or 3μ) and very thick ones (15μ–30μ). These latter were especially useful, and I can strongly recommend the thick-section method to anybody who desires to study the chromosomes of *Aggregata*. The parasites are often so large that even with sections of moderate thickness (*e.g.*, 10μ or so) a single nucleus will be distributed through several serial sections; and consequently, to study the whole of such a nucleus it is necessary to reconstruct it in some way. This is often a matter of no little difficulty, and the process may also introduce an element of doubt into the determination of chromosome numbers. By cutting thick sections, however, these difficulties can be largely avoided, as whole nuclei are thus often obtained in single sections. Even very thick sections (25μ–30μ) can be completely examined with a 2 mm. apochromat if they are mounted under a very thin coverslip (I used coverslips measuring 0·07–0·08 mm. for this purpose): for even with a lens of such short working distance it is possible to focus right through the whole thickness of the section, and to draw the entire contents of a single large nucleus with the *camera lucida*. It is of course necessary to imbed material destined for cutting into thick sections in paraffin of a lower melting point than that used for ordinary sections. (I generally used paraffin of M.P. 37°–40° C. for sections 18μ–25μ in thickness.)

It was often necessary, in the case of the crabs, to section an entire midgut serially, in order to determine whether the animal was infected, or to study all the stages present in it. As the midgut of a fair-sized *P. depurator* measures about a centimetre in length, the labour involved in cutting and examining serial transverse sections of the whole of it is considerable. In order to minimize the labour I therefore adopted a different method, which gave satisfactory results. After fixation and hardening I cut the midgut into halves, transversely; imbedded the two pieces side by side; and then sectioned them together longitudinally—cutting the sections 15μ–20μ thick. A large midgut treated in this way can be cut into comparatively few sections, all of which can be mounted on two or three slides: and it takes far less time to search, with the immersion, through some few scores of such thick sections than through many hundreds of thinner ones.

I stained my material in very many different ways. Indeed, I have stained the sporogonic stages of *A. eberthi* by most of the methods known to me; for as I had, for years, abundant material of these stages always at hand, I generally used it for testing new staining methods. So I will merely mention the best methods here, and will note special points connected with some other methods later.

The best stains for whole parasites, in smear preparations, I found to be Delafield's haematoxylin (strongly diluted), Mayer's haemalum, paracarmine, Grenacher's borax-carmine, alum-carmine, and—especially after Flemming's fixative—safranin. For sections, and especially for studying the chromosomes, I obtained the best preparations with my alcoholic iron-haematein stain[1]; but

[1] See Dobell (1914*a*), p. 143.

other similar stains, such as Heidenhain's aqueous iron-alum haematoxylin, Mallory's iron-chloride haematoxylin, Benda's copper-acetate haematoxylin, and various modifications of these, also gave excellent results. Hollande's iron-chlorocarmine[1], which I used more recently, and Hickson's iron-brazilin, also furnished serviceable preparations. With all of these the usual counter-stains—such as eosin, orange G, light green, Bordeaux red, etc.—were often employed. Of the many double or triple staining methods used, the best results were obtained with safranin and light green, methylene blue and eosin, paracarmine and light green[2], safranin or basic fuchsin and picro-indigo-carmine (Borrel's method), methylblue-eosin (Mann's stain—used in the manner which I have described elsewhere[3]), Mallory's triple stain, Giemsa's stain (wet method, modified). All of these gave good preparations, and were useful for special purposes. Speaking generally, all good cytological staining methods can be used for studying *Aggregata*—provided the original material was really fresh, and really well fixed. Every cytologist likes his own methods best: so I will only add that I prefer, for my own study, whole (smear) preparations stained with dilute Delafield's haematoxylin (progressively) or safranin (after Flemming's fixative), and sections stained with my alcoholic iron-alum haematein (especially after formol-sublimate fixation).

I have devoted so much space to the consideration of technique because it is a matter of paramount importance. It is by no means easy to obtain really first-rate preparations of *Aggregata*; but without them it is impossible to study this parasite properly. Anybody who wishes to repeat my observations must therefore be prepared to spend a long time over it. I suppose I must have had more material at my disposal—for a study of the sexual cycle and sporogony—than any previous worker: but I feel fairly sure also that I must have ruined and rejected far more material than any previous worker has ever possessed. The good preparations from which my ultimate conclusions have been drawn form a very small fraction of the total number that I have, in the course of years, made and examined.

Finally, I must say a word about the optical apparatus which I have used. Nearly all my observations have been made with a Leitz 2 mm. apochromatic homogeneous immersion lens (N.A. = 1·40) or with the 4 mm. apochromat (N.A. = 0·95) of the same maker, and with compensating oculars (2, 6, 8, 12, and 18). I used a large Zeiss microscope fitted with a Leitz aplanatic achromatic condenser (N.A. = 1·40). For illumination I have used a Nernst electric lamp or a "Pointolite" lamp, with appropriate accessories for microscopic work. Monochromatic light was obtained by using Wratten colour-

[1] See Hollande (1916).

[2] This combination I found specially useful for sections of crabs' intestines. I stained the intestines in bulk in paracarmine, and counterstained the sections on the slide with light green in 90 per cent. alcohol. A large number of sections can be rapidly stained and mounted in this way; and after they have been examined particular sections which one may desire to study in more detail can easily be restained with iron-haematein.

[3] See Dobell and O'Connor (1921), p. 137.

screens. All my drawings have been made at an exact magnification, and with the aid of a *camera lucida* and a large adjustable drawing table (Zeiss). It is often very difficult to study the chromosomes at certain stages without drawing them. Even with the best lenses and most accurately adjusted drawing apparatus the task of counting them is often difficult enough; but without such auxiliaries it is, in my opinion, frequently impossible.

In the course of my work I have often found it necessary to "reconstruct" parasites from a number of serial sections. Many are so large that a single section gives an erroneous idea of the whole organism. The method I have used is the simple but effective one of making a series of superimposed drawings of the successive sections with the aid of the *camera lucida*. If the drawings are made from good serial sections, and care is taken to register them accurately by reference to surrounding objects, so that the successive images are correctly superposed, composite pictures of considerable value can be thus obtained. Only a few of the illustrations to this memoir were made in this manner, however, and all of them are duly marked as such in the descriptions of the figures. As a rule, I have purposely figured whole nuclei or chromosome groups from single sections, since reconstruction may introduce an undesirable element of uncertainty.

At first sight *A. eberthi* appears to be an ideal coccidium for detailed cytological investigation. It is very large, possesses enormous nuclei at many stages, and a single section of a sepia's gut often appears to show almost every stage in development simultaneously. But when one comes to study the organism carefully, one finds that its large size is frequently a drawback: for it is too large to examine whole, and, when sectioned, it is difficult to piece together the voluminous nucleus with its complexity of chromatin elements. The abundance of developmental stages usually seen in a single preparation is also deceptive. On examining these stages carefully, it will soon be found that certain developmental forms occur again and again, but that intermediate stages—necessary links for constructing the complete life-history—are almost invariably missing. The very youngest stages in the cuttlefish, and the fertilization stages, are by far the rarest; yet without these it is not possible to understand the rest of the nuclear history. I may say—pour encourager les autres—that I cut, stained, and examined many thousands of sections over a period of five years before I was able to find all the stages in the process of fertilization and reduction—and this notwithstanding that almost every bit of tissue which I sectioned was closely packed with parasites in other stages of development.

Another grievous difficulty arises from the frequency with which abnormal and degenerate parasites occur in almost every infection. No matter how fresh the material may be, no matter how carefully one may have handled it at every step, one always finds abundance of degenerate individuals—at all stages in development—in one's preparations, and they are much commoner in crowded than in light infections: consequently, if one selects heavily infected

tissue for study, in order to obtain the maximum number of developmental stages in one's preparations, one also, as a rule, obtains the maximum number of abnormal and puzzling pictures. It is impossible to describe all these abnormalities here. I will only say that they are very common, often very misleading at first sight, and have therefore often been described and figured by previous workers as normal stages; but that to anybody who has seen the corresponding normal forms—which alone I shall, as a general rule, describe—they are easily understandable and a source of annoyance rather than of deception.

IV. THE LIFE-HISTORY OF *A. EBERTHI*

In the preceding pages it has been noted that the parasites of the genus *Aggregata* display, in the course of their complete life-cycle, the phenomena known as alternation of hosts and alternation of generations. They pass a part of their life in a cephalopod and the remainder in a crab: and in every species which has been studied, the generation in the cephalopod has been found to be a sexual generation, culminating in sporogony, while that in the crab is asexual and ends with the schizogonous production of merozoites.

As no confirmation or extension of the work of Siedlecki (1898) and of Léger and Duboscq (1908) has—apart from my own preliminary notes[1]—hitherto appeared, I propose to record my findings and conclusions in some detail. But I shall first discuss certain general matters concerning the relation of *A. eberthi* to its hosts, and concerning its alternation of hosts and alternation of generations, before I proceed to describe the various stages in its life-history and development.

(A) THE PARASITE AND ITS HOSTS.

(1) *The Cephalopod Host.*

The cephalopod host of *Aggregata eberthi*, as is already well known, is *Sepia officinalis*: and, so far as I have been able to determine, it is this species only. I have examined altogether 330 cephalopods belonging to 14 different species—all from the Mediterranean, and the great majority from Naples—but I have never found *A. eberthi* in any species except *S. officinalis*, in which it is extremely common. Every individual examined at Naples in spring and early summer harboured the parasites, though even such closely related forms as *S. orbignyana* and *S. elegans* were invariably found uninfected. Moreover, from a careful survey of the literature I can find no evidence that anybody else has ever found any species of cuttlefish except *S. officinalis* infected with *A. eberthi*.

The only other cephalopod which is known to harbour *Aggregata* is *Octopus vulgaris*. Early workers, such as Schneider, confused the parasites found in this mollusc with those of *Sepia*: but all competent recent workers are now agreed that the *Aggregatae* occurring in *Sepia* and *Octopus* belong to distinct species. I believe (see below, p. 107) that *S. officinalis* harbours but a single

[1] Dobell (1913), Dobell and Jameson (1915).

species—*A. eberthi*: but how many species occur in *Octopus* I have been unable to determine. Moroff (1908) records no less than eleven species (or possibly even more[1]), for each of which he has a separate name: but it is impossible, from the descriptions which he has published, for anybody else to distinguish most of these species with any certainty. Even Moroff himself cannot distinguish some of them when it comes to the identification of actual specimens: for many of his figures illustrating various stages in the life-histories of these animals are labelled "*Aggregata* sp.?." I have studied the parasites of *Octopus* (at Naples) with some care, and I have spent much time in attempting to reconcile my observations with the statements in Moroff's memoir. But the result has been inconclusive and disappointing. Many of his "species" appear to me to rest upon the fallacious evidence derived from an examination of too small a number of individuals, and from a study of degenerate, abnormal, and imperfectly fixed and stained specimens; and as not a single one of his eleven (or more) species is described at every stage[2], it is impossible for any other worker to interpret all his findings with certainty.

All that I am able to conclude, with confidence, concerning the parasites of *Octopus vulgaris* is (1) that there is more than one species of *Aggregata* in this host; and (2) that, whatever other species these may belong to, they certainly do not belong to *A. eberthi*. The species I found most common in *O. vulgaris* at Naples—where every individual was infected—should be assigned, I think, to *A. octopiana* Schneider. The spores of this species are much larger than those of *A. eberthi*, their average diameter being 14–15 μ: and, in addition to their larger size, they contain far more than three sporozoites (normally about sixteen). At all stages in development the parasites attain a considerably greater size than corresponding forms of *A. eberthi*, and they produce a characteristic and quite different reaction on the part of the host's tissues[3]. The chromosome number and the crustacean host[4] of this species still remain to be determined. On the evidence at present available, I think it probable that Moroff's *A. duboscqi*, *A. jacquemeti*, and *A. reticulosa* were founded upon specimens belonging to this same species: in other words, these three names are probably synonyms of *A. octopiana* Schneider, 1875 (originally placed by Schneider in the genus *Benedenia*, and transferred to *Aggregata* by Léger and Duboscq).

Moroff's species *A. spinosa*, on the other hand, appears to be undoubtedly a good species[5]. It is readily recognizable by the size and form of its spores,

[1] The diagnosis of some of his species is highly unsatisfactory—even the host, in some cases, being left unrecorded, and the parasite (entered as "nov. spec.") being merely said to occur "in the Mediterranean." From other passages in Moroff's memoir I infer that these parasites of marine habitat are supposed to belong to *Sepia*.

[2] Moroff studied the sexual generation only, so that he gives us no information at all concerning the asexual cycle of any of his "species."

[3] See p. 37, *infra*.

[4] Moroff's statements (1907, 1907 *a*, etc.) that *P. corrugatus* is the intermediate host of this (or a similar) species I cannot accept. For he ultimately (1908) produced no evidence of any value in support of it, and I believe that *P. corrugatus* is really one of the natural hosts of *A. eberthi*.

[5] This species was not present in any of the specimens of *O. vulgaris* which I examined at Naples.

the large number of sporozoites in each spore, and the remarkable vermiform karyosome of the adult sexual forms. It seems to me not improbable, however, that Moroff's other "species" from *Octopus* (*A. légeri*, *A. labbéi*, *A. schneideri*, *A. siedleckii*, *A. ovata*, and *A. stellata*) are also referable to *A. spinosa*. At all events, the majority of the forms described under these six specific names do not seem to differ from this species in any important feature—when allowance is made for the fact that many of the stages described and figured are undoubtedly degenerate and abnormal.

In any case there can be no doubt that the species of *Aggregata* occurring in *Octopus* require to be critically revised. From the systematic standpoint they are now in a hopeless muddle, and it seems useless to discuss them further until the facts concerning their real structure and life-histories have been ascertained. I shall therefore say no more here about the parasites of *Octopus vulgaris* and will only emphasize once more the fact that these parasites, though closely related to *A. eberthi*, and undoubtedly undergoing closely similar life-cycles, have no connexion with this species.

The fact that *Sepia officinalis* and *Octopus vulgaris* alone harbour Aggregatidae is doubtless to be explained by the peculiar habits of these two species; and it can hardly be doubted that the freedom from infection of such forms as *Loligo*, *Ocythoe*, and *Rossia*, is closely correlated with their pelagic habits and with the circumstance that they do not usually eat crabs. Whether such animals are incapable of infection, if fed on ripe merozoites of *Aggregata*, remains to be determined experimentally.

(2) *Infection of the Cephalopod Host.*

There can be no longer any reasonable doubt that *Sepia officinalis* acquires its infection with *A. eberthi* by eating crabs (*Portunus* spp.) containing ripe merozoites in their body cavities. The whole sporogony of the parasite occurs in *Sepia*, and the whole schizogony in *Portunus*, and the successful experimental infection of the crab by means of spores from the cuttlefish has already linked up the sporogony and schizogony in unbroken series. Moreover, the merozoites in *Portunus* evidently cannot continue their development unless something occurs which will allow them to escape from the crab's coelome: and the most likely event of this sort to occur would be for the crab to be eaten by some other animal. The chief animal which preys upon *Portunus* is probably *Sepia officinalis*, which appears to be very fond of a diet of crabs: and this is the very animal in which the sporogony of the parasite has been proved to take place. When it is added that the youngest stages of *Aggregata* in the intestinal epithelium of *Sepia* differ but little from the merozoites found in *Portunus*, it is evident that the story is practically complete. Unless some unsuspected stage in development occurs before the merozoites enter the gut of the cuttlefish—a stage unknown in the life-cycle of any other coccidian—we are forced to the conclusion that *Sepia* must become infected with *Aggregata* by eating crabs.

Unfortunately, an experimental proof that this is so cannot yet be given. Nobody—myself included—has been able to perform the experiment of infecting "clean" cuttlefish by feeding them on crabs known to be parasitized by *Aggregata*. The reason why I did not attempt to carry out such experiments when at Naples was because I found that there every individual of *Sepia officinalis* was already infected—and usually heavily infected—with *Aggregata*. As it was impossible to obtain any "clean" animals for experiment—either by breeding them in the laboratory or by catching them in a wild state—I gave up hope of being able to complete my study by infecting cuttlefish experimentally. I hoped, however, that it might be possible to attempt such experiments in some other place where *Sepia* was naturally less heavily infected and where one could therefore rely upon at least a small percentage of wild specimens being uninfected. But when I found later, at Monaco, a place where *Sepia* was not invariably infected, I also found it impossible to obtain sufficient material for experiment, and encountered unexpected difficulty in keeping my animals alive and healthy in the laboratory.

Although every *Sepia* examined by me at Naples was parasitized by *Aggregata*, I thought it would be possible, nevertheless, to obtain some information regarding the earliest stages of development in cuttlefish by feeding these animals upon infected crabs. I thought that if I were to starve the sepias for a few days I could thereby obtain animals from which the very earliest stages of the parasites would be absent. Though I could not obtain wholly clean sepias, I could, I thought, thus obtain sepias whose intestinal epithelium at least would be clean, and in which I might study the earliest stages of infection. I argued that it would be almost impossible to find the earliest stages—in the epithelium—in wild cuttlefish, for it seemed to me highly improbable that I should ever have brought to me a cuttlefish which had just fed upon a well-infected crab at the time of its capture; and consequently, I thought it would be legitimate to conclude that any very early stages which I might encounter in my artificially fed sepias would be referable to my experimental feeding, and not be the results of a natural "wild" infection. But I found that I could not legitimately make such an assumption: for just at the time when I had decided to carry out some experiments along these lines, I examined a recently caught *Sepia* which actually contained some of the very earliest stages in the development of *Aggregata*. This animal (No. 171), though it had been in the laboratory without food for at least 24 hours[1], contained not only early stages of the parasites in the submucous tissues, but even had very young forms—hardly distinguishable from merozoites—within the epithelial cells lining its caecum. It contained, in fact, nearly every stage of *Aggregata*, from almost the youngest in the epithelium up to cysts packed with ripe spores—the result, obviously, of repeated infection in nature.

[1] I am unable to say exactly how long this animal had been in the laboratory, as it was one of several put in the same tank though captured on different days. But it had had no food for at least a day since it was caught, and had not been starved by me for more than three days.

I was therefore very glad that I had not fed this animal on an infected crab whilst it was in my possession. Had I done so, I should certainly have concluded that its youngest parasites were the results of my experimental feeding—a conclusion which might have been erroneous. Sepias infected in this way with very early stages are, it is true, very uncommon: but the circumstance that they may by chance be met with, from time to time, shows how difficult it would be to perform any satisfactory feeding experiments with these animals at a place like Naples, where every individual is more or less heavily infected in a state of nature.

So far as I am aware, the only worker who professes to have infected any cephalopod experimentally, by feeding it upon infected crabs, is Moroff, who states (1908, p. 8) that he infected a *Sepia* by feeding it on *Portunus depurator*. But his experiment will not bear critical examination. He says he fed a *Sepia* (presumably *S. officinalis*, though he does not say so) for 3 weeks upon specimens of *Carcinus maenas* "which were not attacked by *Aggregata*." "Afterwards" he fed this animal "on *Portunus depurator* only." Moroff does not tell us what percentage of the crabs was infected nor what stages of *Aggregata* were present in them—nor even whether they were infected at all. Possibly the *Sepia* was fed only once, for Moroff says that it "died 25 days later"; though nothing is said beyond this to indicate how often the animal was fed upon (supposedly infected) *Portunus*. When this *Sepia* was examined later in "the fresh and fixed condition" (it can hardly have been very fresh if it died naturally and was not killed), it was found to be "very heavily parasitized" with *Aggregata* "in all stages of development." The later stages were, in Moroff's opinion, referable to an earlier infection acquired by the animal before it was captured. This is, no doubt, a justifiable conclusion: but when it is recorded further that "in addition other stages were found, which had quite the appearance of merozoites, and which indicated an infection acquired, at most, two days previously," one cannot accept such a statement without further proof[1]. It is, in fact, merely a guess. Nobody knows how long the earliest stages take in their development; and from such an experiment with an admittedly heavily infected animal, no inference of any value can be drawn. It would be easy to make many such inconclusive experiments, but it is clear that they can supply little or no trustworthy information.

[1] I am fully aware that Moroff (1908) describes (p. 91) and figures (Pl. VIII, figs. 60–66) these "merozoite-like stages" of *A. eberthi*. But he merely calls the earliest of them "merozoites which have just penetrated the gut of *Sepia*," and does not state what cells he found them in. (There is, in his paper, no indication that he ever studied the earliest stages in the intestinal epithelium.) No magnification is given for these figures, and the highly characteristic and remarkable nuclear structure of the earliest stages is not depicted. (Cytological detail of this order was not to be expected if, as I infer, these early stages were obtained from a dead cuttlefish.) Consequently, I am not able to determine exactly how large Moroff's earliest stages were, or precisely what part of the gut wall they came from, or what stage in development they had attained. I will only add that stages as early as those figured by Moroff—whatever stage they may have reached—may be found in wild cuttlefish which undoubtedly acquired their infections in nature.

Whilst I regard it as practically certain, therefore, that *Sepia officinalis* acquires its infection by eating infected crabs of the genus *Portunus*, I have been unable to prove this by conclusive experiments of my own and cannot accept the experimental evidence adduced by Moroff.

(3) *Experimental Determination of the Crustacean Host.*

At the time when I carried out my experiments in Naples, the full results of the work of Léger and Duboscq (1908) were still unpublished. My own work was originally guided by the indications contained in their preliminary notes (1906, 1906*a*, 1907), wherein they stated that the ripe spores of *Aggregata*, from the gut of *Sepia*, will hatch in various crabs. (At this date these workers regarded *Aggregata* as a gregarine, though they still referred the parasites of the sexual cycle in the cuttlefish to the genus *Eucoccidium*.) In their first note (Léger and Duboscq, 1906) they said they had found that the spores of "*E.*" *eberthi* (from *Sepia*) would open and liberate their sporozoites in the guts of *Portunus*, *Pagurus*, *Stenorhynchus*, and *Inachus*: but that in *Portunus* the sporozoites remain motionless, whereas in *Inachus* and *Stenorhynchus* they become very active and penetrate the intestinal epithelium, and later grow into large "gregarines" in the gut wall.

In their second preliminary note, however, Léger and Duboscq (1906*a*) recorded that they had succeeded in infecting *Portunus depurator* (at Cette) with spores from *Sepia*. They found that when this species of crab was fed on ripe spores from a cuttlefish, they all opened "en deux valves" and liberated their contained sporozoites, which rapidly penetrated the intestinal epithelium and then developed into large schizonts in the peri-intestinal lymphoid tissue. These schizonts were those already recognized as belonging to the genus *Aggregata* (then placed among the Gregarines). Some further details concerning the development of the parasite were given in these two notes and in a third which appeared in the next year (Léger and Duboscq, 1907): and it thus seemed clear that, if the observations were correct, the intestinal "coccidium" of *Sepia* and the coelomic "gregarines" of *Portunus*, *Stenorhynchus*, and *Inachus*, were respectively the sporogonic and schizogonic stages of a single sporozoan parasite[1].

Our knowledge of the life-cycle of *Aggregata* was in this somewhat uncertain stage when I began my experiments. Moroff's full paper (1908) appeared whilst they were in progress, but merely served to convince me of the necessity of continuing my work. The admirable final paper by Léger and Duboscq (1908) did not appear, however, until after I had left Naples and had begun to analyse my own results. I mention these points now in order to explain why some of my experiments were performed; for it will be seen that, in the

[1] This parasite, owing largely to the statements then recently made by Moroff (1906, 1906*a*, 1907), was supposed to be not a coccidium—as most early observers of the sexual cycle had supposed—but a gregarine. Even in their final paper Léger and Duboscq (1908) continued to speak of *Aggregata* as a "gregarine," though it is evident that they were not convinced by Moroff's evidence in this connexion.

main, they merely confirm the observations of Léger and Duboscq, as finally reported.

I had a twofold object in attempting to infect crabs with *Aggregata* experimentally. In the first place, I wanted to test the statements made by Léger and Duboscq: secondly, when I found that their account was essentially correct, I wanted to obtain material—of all the stages in development of *A. eberthi* in the crab—for cytological study.

The first point to determine, therefore, was the species of crab which acted as intermediate host for the parasites of *Sepia officinalis* at Naples. I judged, from the observations of Léger and Duboscq, that it would prove to be some species of *Portunus* or *Inachus*: and my preliminary survey of wild crabs (see p. 11) showed me that *P. depurator* was heavily infected in nature with an *Aggregata* (75 per cent.), *P. corrugatus* to a less extent (40 per cent.), and *I. mauretanicus* comparatively seldom (8 per cent.). Consequently, I decided to devote my attention chiefly to *P. depurator*, but to experiment also with *P. corrugatus* and *I. mauretanicus*, and with any other crabs that I could get. Unfortunately, however, *P. corrugatus* was difficult to obtain; and I therefore abandoned work on this species[1], and tried the other two.

The first feeding experiment that I made with *P. depurator* was successful: and in nine subsequent experiments with this species I never had a failure—though I had one doubtful or only partial success to be noted later (p. 33). I was able to confirm the observations of Léger and Duboscq with comparative ease, and I have no doubt that *P. depurator* is the usual intermediate host of *A. eberthi* at Naples. My usual procedure was as follows: Crabs to be experimented upon were kept in the laboratory for several days—occasionally weeks—and fed on fish, from which they obviously could not acquire infection. Before the experimental feeding they were starved for 1–4 days (usually 24 hours only) to remove the food present in their guts. They were then fed on the caeca or intestines of freshly killed *Sepia officinalis* containing abundance of ripe spores of *Aggregata* (ascertained by microscopic examination), which they always consumed with avidity. There was never any difficulty in getting the crabs to feed. Sometimes the feedings were repeated on following days: at other times the crabs were killed shortly after the first feeding, or were given a fish diet again for varying periods before they were finally killed and examined. In this way I was able to obtain all the early developmental stages of the parasite in the crab.

[1] I was the more inclined to leave this species alone because Moroff had stated, on several occasions, that it is the intermediate host of one of the species of *Aggregata* occurring in *Octopus*. He first made the statement through Léger and Duboscq, in a footnote to their first paper (1906), and afterwards repeated it in several of his own works (Moroff (1907 *a*), p. 34, etc.): but the evidence which he was subsequently (1908) able to adduce in favour of this assertion is, to say the least of it, inconclusive. I would point out, on the other hand, that the *Aggregata* which I found in wild specimens of *P. corrugatus* is indistinguishable—so far as my observations go—from that in *P. depurator*: and I am therefore of the opinion that it will prove to be *A. eberthi*, and not one of the species belonging to *Octopus*, if studied again experimentally.

As my observations on *P. depurator* are in almost entire agreement with those of Léger and Duboscq (1908) I will merely note here the general course of events in these experiments, and will then add a few details which seem specially noteworthy. A few hours after the ripe spores of *A. eberthi* have been ingested by *P. depurator*, they open in the intestine and liberate their sporozoites (three from each spore). These become active[1] in the gut contents and soon begin to penetrate the epithelium lining the midgut. Usually within 24 hours, from the time of ingestion of the spores, numerous sporozoites are to be seen in the epithelial cells and in contact with their basal membrane. After a variable period—sometimes several days—the sporozoites pass through the basal membrane into the submucous lymphoid tissue, where they become rounded and grow into schizonts. As they increase in size, the parasites become too large to be accommodated by the comparatively thin wall of the midgut, and they then bulge progressively into the coelomic cavity. When their growth is completed they undergo schizogony, and finally appear as large coelomic cysts attached to the outside of the gut and filled with clusters of innumerable merozoites. At this stage their development in the crab ceases.

According to Léger and Duboscq (1908), crabs are more easily infected during the summer months (May to October) than in the winter. Most of my experiments were made in May and June; and as they were uniformly successful, they agree with the French authors' statements, though they add nothing to them regarding the relative infectibility of crabs at different seasons of the year.

Léger and Duboscq (1908) say that for the first ten days the parasites in the subepithelial tissues of the crab increase in volume without increasing in length—becoming thus rounded, with a diameter of 15–18 μ. Thereafter they increase rapidly in all dimensions, so that they may become fullgrown globular organisms in another 30 days. The complete development of the schizont (resulting in the formation of mature merozoites) requires at least two months. These figures are given for well-nourished crabs during the summer season; and it is stated that development may occur more slowly and with less regularity in winter.

My own observations confirm those of the French workers in so far as the early stages of development are concerned (first 14 days after ingestion of spores). But I was unable to devise any experiments which would enable me to determine the duration of the later stages of development with precision: and for the following reasons. I have previously noted (p. 11) that the wild crabs (*P. depurator*) which I used for my experiments were already naturally infected[2], to the extent of 75 per cent., with *Aggregata*. These crabs were

[1] In their preliminary paper Léger and Duboscq stated that the sporozoites remain motionless in the gut of *P. depurator*: but they corrected this in their full account (1908), and attributed the immobility originally observed to accidental admixture of sea-water with the gut contents in making their preparations. My observations confirm their final statements.

[2] The breeding of "clean" crabs for experiment, in the laboratory, was out of the question: for Dr Lo Bianco and other expert members of the Zoological Station assured me that nobody had ever succeeded in rearing these animals in captivity.

found to contain, when carefully studied by sections, all stages in the development of the parasites from young rounded schizonts (in the subepithelial layer) up to fully mature coelomic cysts filled with merozoites. It was thus clear that I should have to reckon with similar pre-existing infections in many of my crabs destined for experiment. By keeping the animals on a diet of fish for some days, and then depriving them of food altogether for 24 hours or so, I was able to obtain crabs in which the contents of the gut and its epithelial lining were "clean" for experimental purposes: but I could never tell whether any of these crabs might not harbour schizonts in various stages of development in the subepithelial tissues of the intestine, or cysts, in later stages, in the coelome—derived from a previous natural infection. It is impossible to tell whether a living crab is infected with *Aggregata* or not, as the parasites—if present—all lie in the tissues of the gut or in the body cavity[1], and no spores or other stages pass out in the faeces or by any other channel. Consequently, I made no attempt to determine the duration of the later stages of development. I used the material derived from experimentally infected crabs for studying the cytology of the earlier phases of development, and relied on the material obtained from wild crabs for the later stages. I found a sufficient number of overlapping stages in the two series of crabs—those experimentally infected with early stages and those naturally infected with later stages—to indicate beyond any reasonable doubt that I was dealing with the same species of *Aggregata* throughout.

In crabs which were experimentally infected, and examined during the first fortnight, I found ultimately that a previous older infection actually existed in the proportion anticipated. I have absolutely exact information for only 12 such individuals of *P. depurator*, in which the entire midgut was sectioned, and every portion of it closely scrutinized under an oil immersion lens. (In a few cases smears were also made from parts of the intestine, and examined with great care.) The result showed that, apart from the new young infection produced experimentally, a previous infection with older parasites also existed in 8 individuals—which agrees closely with the finding[2] of 21 infected out of 28 wild crabs examined. How Léger and Duboscq were able to determine the duration of the later stages of development I do not know, for they give no information on this point and do not state what percentage of their wild crabs harboured a natural infection—nor even how many they examined. The times which they give seem likely enough, however, and are consistent with my own observations; though I am unable to confirm their statements regarding this particular point for the foregoing reasons.

[1] Coelomic cysts, when present, are easily visible to the naked eye when the crab is dissected. I made several attempts to ascertain whether such cysts were present by operating on living crabs and inspecting their intestines; but animals so treated all died soon afterwards as a result of the operation. Moreover, I soon found that a naked-eye inspection is not sufficient. When young parasites are present in the gut wall, the only sure way of detecting them is by sectioning the entire intestine, and examining every section under a high magnification. A less careful examination, or examination of a part of the intestine only, is inconclusive. [2] Cf. p. 11, *supra*.

In addition to the experiments just described, I performed a few others in order to test whether *A. eberthi* could develop in *Inachus mauretanicus* (= *scorpio*)—as stated by Léger and Duboscq in their earlier communications. The crabs of this species were treated in the same way as the foregoing (*P. depurator*) and the whole of the midgut was sectioned, or teased up and smeared, and every part of it subjected to a most careful examination. I carried out conclusive experiments in this way with only five crabs, but the results were all consistent. None of these crabs was found to have a previous natural infection with *Aggregata*, so that they could all be considered as "clean" for experimental purposes. The results are shown in Table III.

Table III.

Inachus mauretanicus No.	Days when fed on spores (*A. eberthi*)	Day when killed	Result of examination
19	1	2	Many unopened spores in gut. No sporozoites in epithelium. One active sporozoite found in gut contents.
20	1, 2, 3, 4	4 (1½ hours after last feed)	Many unopened spores in gut. Active sporozoites in gut contents. No sporozoites in epithelium.
39	1, 2, 3, 4, 6, 7, 8, 12, 13, 16, 19	20	Some unopened spores in gut, and a few sporozoites. No sporozoites in gut wall or epithelium.
40	Same as No. 39	20	A few sporozoites in gut contents: also a few, apparently degenerate, in epithelium; but none below basal membrane, and no parasites in submucous tissue.
41	Same as No. 39	20	No spores, sporozoites, or other stages discoverable anywhere.

It is clear, from these results, that spores of *A. eberthi* will sometimes open and liberate their sporozoites in the gut of *I. mauretanicus*; but it is equally clear that many spores will not do so, and that infection does not result. It will be seen that the last three crabs experimented upon were fed on spores no less than 11 times in the 20 days during which the experiment lasted. Each received a very large number of ripe spores at each feeding; but as a result only one of the three (No. 40) showed any sporozoites in its gut wall, and these were few and apparently dead, and none of them had penetrated further than the basal membrane[1]. A *Portunus depurator* fed in this fashion would almost certainly have shown a heavy infection, with many stages of the parasites in the epithelial and subepithelial layers of the gut. Consequently, I conclude that *A. eberthi* does not normally develop in *Inachus mauretanicus*. It appears to me probable that the *Aggregata* which occurs in wild specimens of *I. mauretanicus* and *I. dorsettensis* is a developmental form of one of the parasites of *Octopus vulgaris*. It seems unlikely that it can be the species so commonly found in *Sepia*.

[1] Léger and Duboscq (1908, pp. 96, 97) have recorded similar findings in the case of *Inachus dorsettensis*, which they were also unable to infect with *A. eberthi*. They give a figure (Text-fig. 9, p. 97), showing the degenerating sporozoites in the epithelium of the gut, which closely resembles my findings in *I. mauretanicus*.

In their final paper—which was published after I had made the foregoing experiments but before I had fully sectioned and examined my resulting material—Léger and Duboscq (1908) revised their original statements and expressed the opinion that *A. eberthi* will not undergo complete development in "*I. scorpio*" (= *I. mauretanicus*). My results are, therefore, in this respect, again in essential agreement with their ultimate findings.

Although I was convinced that *A. eberthi* was the only species of *Aggregata* which occurred in wild *P. depurator*, I planned some experiments to determine whether the parasites of *Octopus vulgaris* were also capable of developing in this crab. (I thought these experiments important at the time, because Moroff had recently alleged that *Portunus corrugatus*[1] was the intermediate host of "*A. jacquemeti*"—one of the parasites of *Octopus*.) Unfortunately, time was too short for carrying out these experiments, and I had unexpected difficulty in obtaining crabs during the hot weather at the end of my stay in Naples (June 1908); and as a result I was able to feed only two individuals of *P. depurator* (Nos. 55 and 56) on spores of *Aggregata* from *Octopus*. Both crabs were starved for 24 hours, then fed on heavily infected tissue of *Octopus*, and then killed and examined 24 hours later. No. 55 was found to contain no spores, sporozoites, or other developmental stages: but in No. 56 some of the spores had opened and free sporozoites were present in the gut contents, though none were found in the intestinal epithelium. So far as they go, therefore, these experiments furnish no support for the view that the *Aggregatae* of *Octopus* will develop in *P. depurator*, though it is obvious that much further work is necessary to settle the point decisively.

I also intended, had my time allowed, to attempt to infect *Carcinus maenas* with *Aggregata eberthi*—since this Portunid also harbours a species of *Aggregata* according to the observations of Frenzel (1885) and Léger and Duboscq (1908), though I failed to find it in wild *Carcinus* at Naples. But I made the experiment with only a single individual of *C. maenas*, which, although it ingested a vast number of spores from *Sepia*, showed no signs of infection whatsoever when it was subsequently killed and examined.

The results of all my experiments—in so far as the infection of crabs is concerned—may therefore be briefly summarized as follows: *Aggregata eberthi*, whose sexual development occurs in *Sepia officinalis*, undergoes its schizogony in *Portunus depurator* at Naples, where this crab is commonly found infected

[1] I now think it highly probable that Moroff was mistaken, and I believe that the parasite found in wild *P. corrugatus* is identical with that in *P. depurator*. It is quite likely that both species of *Portunus* can serve as intermediate host for *A. eberthi*; for *P. arcuatus*, and probably *P. holsatus*, can also, as Léger and Duboscq (1908) have shown. I would also point out that Moroff's experimental evidence is not above suspicion: for although he says (1908, p. 7)—in somewhat ambiguous terms—that he infected *P. corrugatus* experimentally at Cavalière, he also tells us elsewhere (1908, p. 137) that at this place "all the crabs (*Portunus corrugatus*) are more or less heavily infected" in nature. Further evidence for his conclusions is thus necessary before they can be accepted. For the present I can find no evidence in Moroff's work, or in that of any other writer, to prove that any *Aggregata* of *Octopus* can develop completely in any species of *Portunus*.

in nature, and can be experimentally infected with ease—the parasites developing in it readily, and undergoing their complete schizogony. There is, moreover, definite evidence that *A. eberthi* cannot undergo its asexual cycle in *Inachus mauretanicus*. The spores will sometimes open in this crab, and the sporozoites may even penetrate the intestinal epithelium; but at this stage their development is arrested, and no infestation follows. No evidence was obtained that *A. eberthi* can develop in *Carcinus maenas*, or that any species of *Aggregata* from *Octopus* can develop in *P. depurator*: but these two points were not adequately studied.

Léger and Duboscq (1908) experimentally infected, with *A. eberthi*, two species of *Portunus—P. depurator* at Cette, and *P. arcuatus* at Roscoff. They also infected *P. holsatus*, though in this crab they studied the course of the infection—apparently a normal one—for the first 15 days only. But with *P. puber* their success was only partial, and they believe that in this host *A. eberthi* will not undergo its complete development. The parasites penetrated the wall of the gut, and grew into young schizonts (up to 20μ in diameter); but afterwards they degenerated and died.

Léger and Duboscq (1908) also attempted to infect—but without success—the following other Decapod Crustacea: *Cancer pagurus*, *Carcinus maenas*, *Eupagurus bernhardus*, *E. prideauxi*, *E. cuanensis*, *Homarus gammarus*, *Palinurus vulgaris*, *Pagurus arrosor*, *Stenorhynchus phalangium*, and *Inachus dorsettensis*. They found that when any of these species are fed upon the intestines of *Sepia officinalis* containing spores of *A. eberthi*, some of the spores hatch and liberate their sporozoites: but little or no further development occurs, and no infection is established. All these decapods, therefore, appear to be immune to *A. eberthi*. It must be noted, however, that most of them have been found infected with a species of *Aggregata* in nature. *I. dorsettensis* harbours "*A. inachi*" (Smith, 1905 ; Léger and Duboscq, 1908); *E. prideauxi*, *E. cuanensis*, and also *E. sculptimanus*, harbour "*A. vagans*" (Léger and Duboscq, 1903), as also, apparently, does *H. gammarus* (Léger and Duboscq, 1909): while unnamed species of *Aggregata* have been found in *C. maenas* (Frenzel, 1885; Léger and Duboscq, 1908), and in *St. phalangium* and *Pagurus arrosor* (Léger and Duboscq, 1908). Furthermore, an *Aggregata* named "*A. coelomica*" has been described from *Pinnotheres pisum* by Léger (1901), and another unnamed species is said to occur in *Pachygrapsus marmoratus* (Léger and Duboscq, 1908).

The only certain conclusion which can be drawn at present, from all the known facts, appears to be that *Aggregata eberthi* undergoes its schizogonic development in *Portunus depurator*, *P. arcuatus*, and *P. holsatus*. Probably it can develop also in *P. corrugatus*, but cannot in *P. puber*; while it is almost certainly unable to develop in any of the other crustacea listed at the beginning of the foregoing paragraph. But since many of these crustacea are found infected with a species of *Aggregata* in nature, it is almost certain that some at least—if not all—of these wild infections represent schizogonic stages

belonging to the species of *Aggregata* which occur in *Octopus vulgaris*, since this is the only other cephalopod—besides *Sepia officinalis*—which is known to harbour the sexual and sporogonic stages of Aggregatidae. As it is still impossible to determine how many species of *Aggregata* occur in *Octopus*, and how many different species are distributed among crustacea other than the several species of *Portunus* mentioned, it is useless to attempt to sort out the various forms any further at the present moment.

I have given in Table IV a synopsis of all that is at present known about the occurrence of the various species of *Aggregata* in different hosts. The only species whose whole life-cycle is known is *A. eberthi*. As I hope to be able to show in the present memoir, all the other "species" of *Aggregata* described from *Sepia officinalis* by Moroff (1908) are, in reality, *A. eberthi*, and his names for them are thus merely synonyms of this species. I have therefore excluded them from the table. I must add, also, that I am convinced that many of the "species" described by Moroff from *Octopus vulgaris* are not valid: it seems to me highly improbable that this cephalopod harbours 11 species of *Aggregata*[1], so I give Moroff's names for what they are worth. It seems certain, however, that *Octopus* harbours two or more species of *Aggregata*; and the conclusion appears irresistible that some, at least, of the parasites from this cephalopod must be stages of some of those which occur in crustacea, but of which the cephalopod hosts are still undetermined.

Table IV.

	Host		
Parasite	Cephalopod (sporogony)	Crustacean (schizogony)	Observer
Aggregata eberthi (Labbé) L. & D.	*Sepia officinalis*	*Portunus depurator*	Léger & Duboscq 1906, 1908; C.D.
		P. arcuatus	Lég. & Dub. 1908
		P. holsatus	Lég. & Dub. 1908
		P. corrugatus (?)	Lég. & Dub. 1908; C.D.
Aggregata coelomica Lég.	?	*Pinnotheres pisum*	Léger 1901; Lég. & Dub. 1908
Aggregata vagans Lég. & Dub.	?	*Eupagurus prideauxi*	Lég. & Dub. 1903, 1908
		E. cuanensis	Lég. & Dub. 1903, 1908
		E. sculptimanus	Lég. & Dub. 1903
		Homarus gammarus (?)	Lég. & Dub. 1908, 1909
Aggregata inachi Smith	?	*Inachus dorsettensis*	G. Smith 1905; Lég. & Dub. 1908
		I. mauretanicus (= *scorpio*)	G. Smith 1905; C.D.
Aggregata sp.	?	*Carcinus maenas*	Frenzel 1885; Lég. & Dub. 1908
Aggregata sp.	?	*Pagurus arrosor*	Lég. & Dub. 1908
Aggregata sp.	?	*Stenorhynchus phalangium*	Lég. & Dub. 1908
Aggregata sp.	?	*Pachygrapsus marmoratus*	Lég. & Dub. 1908
Aggregata octopiana Schn.	*Octopus vulgaris*	?	Aimé Schneider 1875, *et alii*
Aggregata spinosa Moroff	*Octopus vulgaris*	?	Moroff 1906*a*, 1908
"*Aggregata jacquemeti*" Mor.	*Octopus vulgaris*	*Portunus corrugatus* [??]	Moroff 1906, 1908
"*Aggregata duboscqi*" Mor.	*Octopus vulgaris*	?	Moroff 1908
"*Aggregata reticulosa*" Mor.	*Octopus vulgaris*	?	Moroff 1908
"*Aggregata légeri*" Mor.	*Octopus vulgaris*	?	Moroff 1908
"*Aggregata labbéi*" Mor.	*Octopus vulgaris*	?	Moroff 1908
"*Aggregata schneideri*" Mor.	*Octopus vulgaris*	?	Moroff 1908
"*Aggregata siedleckii*" Mor.	*Octopus vulgaris*	?	Moroff 1908
"*Aggregata ovata*" Mor.	*Octopus vulgaris*	?	Moroff 1908
"*Aggregata stellata*" Mor.	*Octopus vulgaris*	?	Moroff 1908

[1] Cf. p. 20.

(4) *The Immunity of certain Crustacea to Infection with* Aggregata.

It is now necessary to say something more about the "immunity" of certain crustaceans to infection with *A. eberthi*. One of the striking phenomena brought to light by Léger and Duboscq (1908) is the infectibility of crabs of the genus *Portunus*: but equally striking, perhaps, is their discovery that other crustacea are incapable, or only "partially capable," of infestation by this parasite. The immunity, or partial immunity, of such animals presents several features of interest, and these, although they have already been touched upon, deserve further consideration. I shall therefore summarize the chief facts relating to this subject at this juncture.

It has been established that the spores of *A. eberthi* (from *Sepia*) will not open and liberate their sporozoites in the intestinal juices of their cuttlefish host. (This has been shown by Léger and Duboscq—*contra* Siedlecki—whose observations I can confirm.) But they will develop in this way in the intestinal juices not only of crabs of the genus *Portunus*—their natural hosts—but also in a variety of other crustacea (shore crabs, hermit crabs, spider crabs, lobsters, etc.), some of which are not very closely related. In some cases the sporozoites are even capable of invading the intestinal epithelium, and going through the earliest stages of their development: but they soon die and degenerate, so that no infestation results. Such a "partial infestation" is well seen in the case of the spider crabs, *Inachus dorsettensis* (Léger and Duboscq, 1908) and *I. mauretanicus* (as described in the present paper, p. 28). Léger and Duboscq have concluded that "immunity" to infection, in such cases, is due to the toxicity of the crab's tissues for the sporozoites; and it obviously cannot be due to the incapacity of the spores to hatch, or to the inability of the sporozoites to penetrate the tissues. In other cases, however, this last factor must also be taken into account; for in most of the "immune" species the sporozoites, though liberated in the intestine, never penetrate the epithelium, and ultimately pass out with the faeces and perish. Both these degrees of immunity may, indeed, be manifested by different individuals of the same species. (For example, in *Inachus mauretanicus*; see my experiments with Nos. 39, 40, and 41, p. 28.)

The case of *Portunus puber* (noted on p. 30) is also noteworthy. Léger and Duboscq were never able to infect this species completely, though all other species of the genus hitherto tried appear to be readily and completely infectible. In *P. puber*, however, infection aborts at a later stage than in *Inachus*, for the parasites may enter the subepithelial tissue and develop into young schizonts before they die.

But even *Portunus depurator* itself appears sometimes to possess, or to be able to acquire, some degree of immunity to infection with *A. eberthi*. Léger and Duboscq have recorded instances in which the parasites, instead of growing normally, degenerated and died, and finally fell a prey to phagocytes. They discuss such phenomena at some length, and they point out—*inter alia*—

that the basal membrane of the intestinal epithelium may sometimes act as a barrier to the entry of the sporozoites. I should like to mention a few of my own observations in this connexion.

I have already noted (p. 25) that I had one possible failure in my attempts to infect *P. depurator*. This was in the case of crab No. 30. This animal—prepared for experiment as already described—was fed upon immense numbers of ripe spores of *A. eberthi* (from three different cuttlefish) upon the first, third, and fourth days of the experiment. It was killed on the fifth day, and the whole of its midgut—except a small bit of the extreme anterior end, which was fixed and stained as a smear preparation—was preserved and sectioned. Examination showed that although the sporozoites had been liberated as usual, not a single one had passed beyond the basal membrane of the intestinal epithelium, and even in the epithelium itself they were scarce and difficult to find. The basal membrane was very thick, and from the appearance of the sections it was clear that it had effectively blocked the entry of the sporozoites. As one would expect to find young parasites in the subepithelial layer on the fifth day, and as the crab had ingested a very large number of ripe spores at each feed, I was surprised at the result of the experiment. It is possible, from the findings, that this crab might not have acquired anything more than a transient infection of the intestinal epithelium—like *Inachus*—and I am consequently in doubt whether I succeeded in "infecting" it or not. It should be added that the sections also revealed the presence in this crab of a much older natural infection with *Aggregata* (coelomic cysts containing merozoites).

I obtained a somewhat similar result with another *P. depurator* at about the same time. This animal (No. 29) was fed on the first, second, third, fifth, sixth, seventh, ninth, eleventh, and twelfth days of the experiment (*i.e.*, nine times in 12 days) with ripe spores from nine different sepias, and was killed on the thirteenth day. Its whole midgut was sectioned and carefully examined; but instead of finding, as was to be expected, a heavy infection with all the early stages, I found only a very few extremely young schizonts in the subepithelial tissue. But the epithelium was crowded, in places, with sporozoites—evidently completely blocked by the unusually thick basal membrane. From the appearance of the sections (see Fig. 169, Pl. VI) I have no doubt that the very small infection of the subepithelial tissue in this case was the direct result of the inability of the sporozoites to pass through the basal membrane, which thus conferred an effective immunity upon its possessor. The sporozoites found must have been derived from spores eaten during the last few days of the experiment. Those from the earlier feedings had all disappeared, with the exception of the very scarce parasites in the submucous tissue. Those found in the epithelium could hardly have survived for very long; and in spite of the very intensive feeding upon spores the crab would thus, had it lived, have acquired only a very small infection ultimately—so small as to be out of all proportion to the immense number of sporozoites which had been

liberated in its gut. In this crab also there was evidence of a much earlier natural infection (coelomic cysts with merozoites).

As both these crabs (Nos. 29 and 30) were animals which—when wild—had been previously infected with *Aggregata*, it might be argued that they were individuals which had acquired some immunity. There is, however, little or nothing to justify such a supposition; and it should be noted that in my other experiments the existence of an earlier infection never seemed to have any influence upon the infectibility of the host. Repeated infections were easily obtained, and in this respect my observations are evidently in accord with those of Léger and Duboscq (1908).

I have no doubt that the sporozoites are usually able to penetrate the basal membrane and so pass into the submucous tissue, for I have seen all stages in this migration in my preparations. But I am also convinced that, when the basal membrane happens to be unusually thick, it does act as an effective barrier to the entry of the sporozoites. Immunity, in such cases, is therefore purely mechanical or "morphological."

I did not observe any notable difference in the intensity of infection in wild crabs (*P. depurator*) of different ages; but I did not examine a sufficiently large number of individuals of different ages to ascertain with certainty whether age is correlated in any way with degree of infection. I mention this point because Moroff (1908, p. 139) has stated that "the intensity of infection in *Portunus corrugatus* appears to decrease with increase in age," the evidence for this being that he found heavy infections in young crabs, but "in the guts of much bigger crabs, only isolated cysts or often, indeed, none at all." This observation was made at Cavalière: but how the last statement just quoted is to be reconciled with the same writer's remark (1908, p. 137) that "all crabs (*P. corrugatus*) are more or less heavily infected" in the very same locality, I do not know. From his observations—whatever they were—Moroff concludes (1908, p. 139) that the older individuals of *P. corrugatus* have probably "acquired an immunity, up to a certain point"—a conclusion which appears to me questionable.

(5) *The Natural Infection of* Portunus.

The experimental infection of crabs in the laboratory has been hitherto achieved by feeding these animals upon pieces of the fresh gut of a cuttlefish containing ripe spores of *Aggregata*. In this way, one can be certain that the crab gets a sufficient quantity of spores to produce an evident, and often massive, infection. But it is unlikely that crabs usually become infected in this manner in a state of nature. *Sepia* preys upon *Portunus*, and not *Portunus* upon *Sepia*; and consequently it is hardly justifiable to assume that *Portunus* always acquires its natural infection by consuming the living tissues of a *Sepia*. How, then, does the crab normally get infected?

Léger and Duboscq (1908, p. 52) believe that crabs usually acquire their infections in nature by eating the faeces of cuttlefish. As is well known,

P. depurator is a scavenger, and will eat almost any kind of animal matter. In the laboratory I find it will greedily devour (when hungry) any part of a dead cuttlefish—except the ink-sac—which is offered to it. It does not care, apparently, whether its food is fresh or in an advanced state of decay, and it will readily ingest the whole gut of a *Sepia*—contents and all. It is therefore probable that a wild *P. depurator* would ingest the faeces of cephalopods if it came across them. But it is rather remarkable that neither Léger and Duboscq nor any other workers appear to have tried to infect crabs by means of the faeces of cuttlefish: indeed, I can find no definite statement that any worker has even examined the shed faeces of *Sepia* to ascertain whether they really do contain spores, as the hypothesis requires.

I must note here, however, that Moroff (1908, p. 132), makes some pertinent statements on this subject. He says that the natural infection of crabs is brought about by their ingesting "the faeces of the appropriate cephalopod, in which ripe cysts are present in sufficient quantity," and he goes on to remark that "artificial infection is very easily achieved. One has only to feed crabs with the infected gut of a *Sepia* or an *Octopus*." But I cannot find any evidence in these statements to show whether Moroff ever examined the *faeces* of cephalopods in order to see whether they really contain "ripe cysts"—though he makes this assumption. If he did, he gives us no further information about his observations, nor does he tell us how these cysts—which are formed in the submucous layer of the gut wall—manage to get into the faeces[1]. Moreover, his final remarks about experimental infection are clearly not to the point. It is perfectly true that crabs can be readily infected experimentally by feeding them on the *tissues* of the gut containing ripe spores—as Léger and Duboscq showed: but this is no proof that crabs acquire their infections in nature by feeding upon the *faeces* of cephalopods.

As I have been able to find no satisfactory statements on this subject in previous accounts of *Aggregata*, I shall therefore record here a few of my own observations bearing upon the matter. Although they are unfortunately incomplete, they supply definite information on several points.

I have found, in the first place, that the spores of *Aggregata* do pass out of the intestine of *Sepia* with its faeces. In the bulk of the faecal mass, however, they may at times be difficult or impossible to find—even when the *Sepia* is known, from subsequent dissection, to have been heavily parasitized. Careful examination of sections of the intestine shows—as Siedlecki and others have observed—that oocysts filled with masses of ripe spores may rupture into the lumen of the gut. This involves some destruction of the mucous membrane, since the oocysts all lie in the submucous tissues. But in a

[1] Elsewhere in his memoir Moroff says (1908, p. 139) that "the ripe cysts get into the lumen of the gut, whence they are discharged to the exterior": but this appears to be a matter of belief rather than of observation. The same author also tells us a little later (1908, p. 140) that "the ripe cysts are scattered broadcast through the water," but here, also, he adduces no evidence in support of his assertion. For my part, I have never found spores of *Aggregata* in the sea, and I think it would be very difficult to discover them there—if present.

heavily infected cuttlefish the destruction of mucous membrane may be considerable[1]. There is sometimes an extensive necrosis and ulceration of the lining of the gut, and much dead epithelium is then shed into its lumen. A catarrhal condition results and is outwardly manifested by the cuttlefish passing rags and tatters of necrotic intestinal mucous membrane *per anum*. These rags, or sloughs, when examined under the microscope are seen to consist of mucus and tissue débris (from the lining of the gut), often packed with spores of *Aggregata*. I have seen a *Sepia* pass a strip of necrotic tissue of this sort 5 or 6 cm. in length—approximately as long as the animal's intestine—and this slough, under the microscope, appeared as an almost solid mass of spores. It is probable, I think, that the spores of *Aggregata* normally emerge from the cuttlefish in this manner[2]: the epithelium of the gut undergoes necrotic changes and is cast off; the spores in the subepithelial tissues are then liberated; and the sloughs so formed, containing numerous spores, are finally shed into the lumen of the gut and pass out with the faeces.

It seems to me probable that *P. depurator* usually acquires its infection in nature by eating such spore-containing sloughs in the excrement of a *Sepia*. It will certainly eat such sloughs, when it can get them, in the laboratory; and by doing so it can probably become infected—as the two following observations show.

(1) One day I put a small *P. depurator* in a large tank containing a hungry *Sepia officinalis*. I expected to see the cuttlefish eat the crab: but instead of attacking it the cuttlefish continued to swim about unconcernedly near the surface. As I was watching the *Sepia* it suddenly discharged a ragged whitish shred of matter from its funnel. This shred was some 3 cm. or so in length, and it floated in the water for a few minutes and then began to sink flimsily towards the bottom. Before it had gone far, however, the *Portunus* caught sight of it, and leaving the corner of the tank—to which it had retired—it swam[3] up towards the shred, seized it, and began to cram it into its mouth. Fortunately, I was able to catch the crab before it had swallowed the whole of its capture: and on examining a bit of this under the microscope, I found that it was—as I suspected—a slough of necrotic tissue containing spores of *Aggregata*. I put the crab in another tank, and killed and examined it next day. It contained hatched spores and sporozoites in its gut, and would doubtless, therefore, have acquired an infection with the parasite if I had kept it longer.

(2) On another occasion, as I was watching a large *Sepia* disporting itself in its tank, I saw it suddenly eject what looked at first sight like a long white half-disintegrated worm, about 5 cm. in length. I removed this object—which remained afloat—with forceps, and examined it under the microscope. As

[1] In a slight infection the destruction of tissue is, of course, trivial. The description given above refers only to those cases where the infection is intense, and where the effects are therefore conspicuous.

[2] The oocysts also rupture and liberate their spores into the submucous tissue, where they may eventually be consumed by phagocytes.

[3] *P. depurator*, though a scavenger on the sea-bottom, is an excellent swimmer.

before, I found it was a slough containing abundant spores of *Aggregata*. I gave the whole of this to a *Portunus depurator*—in another tank—which promptly devoured it. I intended to keep the crab for some time to see whether it would become infected: but a few days later it escaped from its tank in the night, and when I recovered it the following day it was dead. The microscopic examination was therefore unsatisfactory, but it showed the presence of sporozoites—though apparently dead—in the intestinal epithelium.

Unfortunately, I was not able to repeat the foregoing experiments, for they require peculiar conditions and a certain amount of luck. But even the few observations which I have been able to make show clearly, I think, how a crab may acquire infection in nature. They show that spores of *Aggregata* do occur in the discharges from infected sepias, and that *P. depurator* has no distaste for the droppings of these animals, but on the contrary will ingest them readily, and may thereby become infected.

It is probable that crabs also get infected by eating the bodies of dead cuttlefish. It is easy to infect them in this way in the laboratory: the only doubt is whether, in nature, crabs encounter dead cuttlefish sufficiently often to account for their high rate of infection. Léger and Duboscq (1908) point out, however, that dead sepias must at times be fairly plentiful in the sea: and they relate—on the evidence of the laboratory keeper at Roscoff—how, at certain seasons, the porpoises attack the cuttlefish, eat their heads, and leave their dead bodies floating as a prey for the crabs. The same writers also note that fishermen use cuttlefish as bait for their traps, and frequently cast their dead remains into the sea.

From all this it seems justifiable to conclude that *Portunus* may acquire its infection in nature either from eating the excrement of cuttlefish or by devouring the dead bodies of these animals. Both methods would, probably, be equally effective; but at present it is impossible to say which is the more usual.

(6) *The Effects of the Parasite upon its Hosts.*

I have already had to note some of the effects of *A. eberthi* upon the tissues of its hosts, but a few additional points remain to be considered.

Sepia officinalis is often very heavily infected with *Aggregata*. Almost the whole of the subepithelial tissue of its caecum, and the upper part of its intestine, may at times exhibit an infestation with parasites packed so closely together that they squeeze one another out of shape and inhibit one another's development. In such sepias the submucous tissue appears to be largely replaced by parasites. Yet even in such heavily infected animals the reaction on the part of the tissues appears to be but slight, with the exception of the periodic sloughing or "moulting" of the epithelium which has already been described. Invaded cells merely die and degenerate: they show no other changes, and in this respect they differ conspicuously from the cells of *Octopus vulgaris* invaded by *A. octopiana*. The invaded cell, in this host, undergoes an enormous hypertrophy, in which its nucleus shares; and the appearance

of such overgrown cells with their vast nuclei, surrounding parasites at all stages of development, is so characteristic that one can usually tell at a glance whether a given section of the gut of a cephalopod parasitized by *Aggregata* was derived from *Sepia* or *Octopus*. In Brumpt's *Précis* (1913, p. 44) there is a picture (Fig. 21) which is said to have been drawn from a section of the gut of *Sepia* infected with *Aggregata*: but as it shows the typical tissue-reaction seen in *Octopus*, I have little doubt that it was really drawn from a preparation of the intestine of this animal—not *Sepia*[1]. At all events, I have never seen, in all the material from *Sepia* which I have examined, a hypertrophy of the infected cells such as Brumpt figures. If it occurs, it must be extremely rare—though it is common enough to be regarded as "normal" in *Octopus*.

The effects of *A. eberthi* upon the tissues of *Sepia* appear to me to be almost purely mechanical. When the gut wall is packed with parasites it becomes thickened and deformed, and its tissues are compressed to such an extent that their normal cellular structure may be completely obliterated. Masses of parasites in the submucous layers must cut off or impair the blood supply to the mucous membrane; and it is this, no doubt, that leads to the degeneration and death of the epithelial cells and the formation of the intestinal sloughs already described. The sloughing of the mucous membrane may involve only a minute area and a few epithelial cells, or it may involve larger and larger areas—sometimes almost the whole lining of the gut. Only very large sloughs are conspicuously visible when passed *per anum*: but I have no doubt that every infected *Sepia* constantly passes microscopic shreds of mucous membrane and small sloughs or aggregations of dead epithelium with its faeces, though these, as a rule, would not be observable to the naked eye. Very large sloughs, such as I have described, are the exception and not the rule; but they show, in a striking manner, the events which must be constantly happening on a much smaller scale throughout the life of a parasitized animal.

In almost every infection many dead and degenerate parasites are to be seen. They are to be found in even the freshest and best preserved material; and although they are more numerous in very crowded infections, they also occur in lightly infected areas of the intestine where the parasites are widely separated. The parasites may die and degenerate at any stage, and their disintegrated remains—often containing granules of a brown pigment—then become dispersed through the submucous tissue and are attacked and eaten by phagocytes. Ultimately the space occupied by the parasite is filled up with new connective tissue. When the oocysts, at the end of development, fail to discharge their contained spores through a breach in the mucous membrane into the lumen of the gut, they break down *in situ* in the wall of the intestine, and the spores are thus scattered in all directions. Large areas of submucous tissue infiltrated with loose spores may often then be seen. Many of these

[1] Brumpt's other figure of *Aggregata* in *Sepia* (Fig. 25, p. 45) shows, on the other hand, the appearance typical of this genus, and is therefore, I believe, correctly labelled.

spores ultimately die and disintegrate, but some at least are ingested and destroyed by phagocytes in the gut wall.

It is noteworthy that neither very intense infestation nor even very extensive sloughing of the mucous membrane seems to cause the cuttlefish any inconvenience. An animal which appears to have more parasites than submucous tissue in its gut wall, or one which has, apparently, sloughed off the greater part of the lining of its gut, will continue to live and perform all its usual functions as though it were perfectly healthy and normal. The regeneration of lost and destroyed tissue occurs, I believe, very rapidly; for although I have made no detailed study of this process, I several times observed that animals which had previously "moulted" much mucous membrane appeared to have a normal and intact intestinal lining when dissected a few days afterwards.

As every specimen of *S. officinalis* which I examined at Naples was infected with *Aggregata*, I can only suppose that infection with this parasite is the "normal" condition of these animals in nature—at any rate during spring and early summer in the Bay of Naples. It is therefore impossible to believe that the parasites exercise any really harmful effects upon their hosts.

The effects of *Aggregata* upon *Portunus* have been well described by Léger and Duboscq (1908), to whose observations I can add nothing new. I will merely note here that in the crab, as in the cuttlefish, even very heavy infestation with parasites appears to produce no harmful effects. In the crab's gut the tissue-changes which are associated with moulting make a study of the damage done by the parasites peculiarly difficult; but it seems certain that, as a rule, the displacement of the subepithelial tissues, and the blocking up of the body-cavity resulting from heavy infestation, cause the host very little inconvenience.

According to Geoffrey Smith (1905), "*Aggregata inachi*" causes the "parasitic castration" of its host (*Inachus dorsettensis*). Although Smith speaks very confidently[1] regarding this phenomenon, I am not convinced by the evidence which he has published. Smith believed that *Aggregata* was a gregarine, and he appears to have supposed—incorrectly—that the final stage of its development in the crab was the liberation of the merozoites ("sporozoites") into the body cavity, where—in some cases at least—they produced "in some unknown manner" the dissolution of the testis.

According to Smith "more than half the specimens" of *I. dorsettensis* which he collected at Naples were parasitized by *Aggregata*, though he gives no more precise figure for the incidence of infection. But he notes that his parasitized individuals "contained a great number of cysts"; and as small infections can only be detected by very laborious microscopic investigation,

[1] In the title of his paper, it is true, Smith refers to the parasite as one which "*may* cause" castration. But it is clear, on reading the paper itself, that the author had no doubts about the matter. By "may cause" he did not, apparently, wish to imply that there is any doubt about the reality of the process, but meant "can cause, but does not invariably do so," as is evident from his statement that many of his parasitized animals were not castrated.

it must therefore be assumed that probably far more than 50 per cent. of his wild material was actually infected. Out of 50 males examined, seven presented externally a "hermaphrodite appearance," and on dissection were found to contain cysts of *Aggregata*; while "the body cavity was also full of liberated sporozoites" and "the testes were in all cases disintegrated." When the apparently high rate of infection of "normal" individuals is remembered, it does not seem surprising that all seven of these animals should have been found infected. I have never seen a crab with its body-cavity in the state described by Smith, but it does not seem to me unreasonable to suppose that whatever it was that caused the disintegration of the testes also caused the disintegration of the cysts of *Aggregata* which happened to occupy the body-cavity at the same time. At all events, no evidence is brought forward to prove that the parasites in any way caused the disintegration of the testes; and the disruption of the cysts—which, contrary to Smith's assumption, does not normally occur—still remains to be accounted for. When it is noted further that the "sporozoites" which were found in the body-cavity were, to judge from the figures, not merozoites at all, but cells belonging to the body of the crab, the interpretation of these observations seems extremely dubious.

From his observations Smith concluded that "It is impossible to prove from the instances before us that the presence of the parasite is the cause of the castration, but that this is the case is certain from the analogy of other instances." With the first part of this statement I entirely agree; but I confess that the second part appears to me both illogical and unwarranted.

I have not studied the *Aggregata* which occurs in *Inachus dorsettensis*; but I can state with some confidence that I have been able to find no evidence, from my own observations or those recorded by Léger and Duboscq, which would warrant the inference that *A. eberthi* causes the parasitic castration of *Portunus*. So far as I have been able to ascertain, parasitized individuals display no structural characters, external or internal, which differentiate them from normal uninfected specimens. I am confident, moreover, from an examination of the gonads, that *Aggregata* did not cause the parasitic castration of the specimens of *Sepia officinalis* which I have studied.

(7) *Alternation of Generations in* Aggregata.

As already noted, alternation of hosts is associated, in *Aggregata*, with alternation of generations. The cycle of development undergone by the parasite in its cuttlefish host is entirely different from that which it passes through in the crab. As the facts are now perfectly clear, they call for no discussion: but I would lay stress upon the two following points, since they are of great importance for a proper understanding of the life-cycle of *A. eberthi* —a subject which I shall have to consider in detail in the next section.

First, it must be emphasized that the development of *A. eberthi* in *Sepia officinalis* is sexual and sporogonic. The parasites in this host are differentiated into males and females—producing microgametes and macrogametes respec-

tively. Fusion of these gametes produces a zygote which is a sporont—giving rise to a large number of resistant spores containing sporozoites. The whole of this sexual cycle follows the lines familiar to all students of the Coccidia. The development in *Portunus depurator*, however, is entirely asexual. In this host the sporozoites, liberated from their spores, develop into schizonts which divide by schizogony (multiple fission) to produce large numbers of merozoites: and here again the development—speaking generally—is along familiar lines. The point which I wish to emphasize is that the two cycles of development—sexual and asexual—are rigidly confined to their respective hosts. No asexual development ever occurs in *Sepia*, and no sexual development in *Portunus*. It is thus impossible for any observer, who knows the provenance of his parasites, to confuse stages belonging to the two different cycles.

Secondly, I wish to emphasize the fact that the developmental events in either host—whether cephalopod or crab—form a single unbroken and unrepeated series. Each male and female parasite goes through a continuous series of changes culminating in gamete formation, and after fertilization another single series of events leads to the final production of spores. When the spores are formed, the development of the particular parasite concerned is at an end—until it reaches an entirely different environment. There is no recurrence of the sexual cycle. The same holds true of the asexual phase. Schizogony occurs once, and once only, in a given parasite in a given crab. The merozoites are incapable of developing into schizonts and repeating the asexual cycle in the same host. A merozoite, when once formed, can undergo no further development unless it can enter an entirely different host.

These two points are of very great practical importance. In most Coccidia the sexual and the asexual cycles occur in the same host, and in the same site in that host, while the asexual development usually recurs several times. In *Aggregata* Nature has not only simplified matters for the cytologist by separating the two cycles with the utmost precision—putting one in one host, the other in another—but she has also further simplified matters by avoiding a repetition of events at any stage in the life-history. These circumstances, accordingly, are of inestimable value to the investigator; for they eliminate, from the start, a number of possible causes of error in interpretation—errors which are, in the study of most other Coccidia, almost unavoidable.

(B) THE DEVELOPMENT OF *A. EBERTHI*.

I shall now describe as briefly as possible the development of *A. eberthi* throughout its whole life-cycle. In doing so I shall chiefly record my own observations and conclusions, and as a general rule I shall not refer to the works of others unless priority in the discovery of any particular phenomenon is in question, or unless their descriptions or figures seem to me to require special comment. I have read with care the works of all previous writers on this subject—of all, that is, whose existence is known to me, after diligently searching the literature—and I have profited by their experience. If I do not

discuss every detail in their records, it is from no wish to belittle or disparage their works, but merely for the sake of brevity.

I would note also, at the outset, that in my descriptions I shall generally consider the normal development only. To describe all the abnormalities which I have studied would double the length of my account: and if I do not mention certain forms which others have described, it is usually because I have satisfied myself that they are abnormal or degenerate stages, or results of faulty technique or interpretation.

Almost every stage in the life-history of *A. eberthi* has been described, more or less, by some previous worker. Nevertheless, many important details have been overlooked, and no complete description of the whole life-history has yet been published by any one observer. The most accurate accounts at present available are those of Siedlecki (1898) for the sexual cycle and of Léger and Duboscq (1908) for the asexual: and as a general rule I shall not figure stages which have already been adequately depicted by these workers[1].

(1) *The Earliest Stages in* Sepia officinalis.

The youngest stages of *A. eberthi* in *Sepia* I have found in the epithelium of the caecum[2] (Pl. I, fig. 1). In structure they differ slightly from the merozoites, from which they are derived, but are of approximately the same size—being minute sausage-shaped organisms about 9–11 μ in length and 2 μ in breadth. When stained and highly magnified they show (Figs. 1, 8) a relatively large and nearly central nucleus, with a peculiar structure. The organism itself is somewhat curved, with one of its ends more pointed than the other—as in the merozoite (cf. Pl. I, fig. 8 and Pl. VI, fig. 198). Sometimes the remains of the mucron of the merozoite is still visible at the pointed end, and the vacuole and axial filament can still be made out (Fig. 8): but these structures do not long persist.

The merozoites penetrate the epithelial cells by their own activity, and bore their way through the cytoplasm. (I have not seen them doing so, when alive; but I have seen living merozoites moving actively and I have been able to trace them, when fixed and stained, right through the epithelium.) When inside an epithelial cell the young parasite always lies in a vacuole, in stained preparations (Fig. 1). Probably it passes through the epithelium rapidly, for intra-epithelial stages are rare, and very young parasites—which have undergone but little growth and differentiation—are to be found in the subepithelial tissues. Occasionally a young parasite is arrested in the epithelium. It may stay there and grow into a young rounded form; but it is unable to complete

[1] The reader who is unfamiliar with the life-cycle of *A. eberthi* will find it helpful to refer at this point to the summary of the life-history on p. 124, *infra*, and to Text-fig. 3, p. 125.

[2] They must also occur in the epithelium of the intestine, since the later stages are commonly found in this part of the gut: but I have not succeeded in finding the very earliest stages in this situation. The youngest stages in the epithelium were first figured by Mingazzini (1893): but his pictures (Pl. I, figs. 2–5) are very small, and not accurate.

its development in the mucous membrane, and such arrested organisms[1] ultimately degenerate and die *in situ*.

The merozoites, after traversing the epithelium and its basement membrane, reach the submucous connective tissue, where they come to rest and begin to develop. But although I have seen abundance of parasites, at all stages, in the connective tissue, I have found it extremely difficult to determine their exact habitat; and I am now convinced that their relations to the host are not always the same, but may be established in any one of three different ways.

(1) Sometimes—and this appears to be most usual—the merozoite enters a connective tissue cell (Pl. I, fig. 9), and stays in this (or its degenerated remains) for the rest of its life. The parasite lies in the cytoplasm—never in the nucleus—and as it grows inflates its host-cell to such an extent that it becomes a mere membranous investment (Fig. 11). The nucleus of the host-cell sometimes becomes slightly hypertrophied[2], and is later flattened over the surface of the parasite. Finally it degenerates with the rest of the cell, though both contribute to the formation of the capsule with which every parasite is surrounded in its later stages. As the parasite grows, it displaces the surrounding connective-tissue cells, which become flattened out and irregularly plastered on its surface. Sooner or later these cells degenerate, and become adherent to the remains of the original host cell. The final result is that the parasite lies intra-cellularly but surrounded by an additional capsule formed from a variable number of degenerate connective-tissue cells.

(2) Sometimes the parasite develops intercellularly—not intracellularly. It begins to grow in an intercellular space in the submucous tissue. But it soon becomes invested with a layer of flattened connective-tissue cells—as in the former instance—and the capsule finally formed is similar in appearance. It is multicellular and surrounds and adheres to the organism; but though the organism is thus imbedded in the tissues, it does not lie inside a cell.

(3) Sometimes—but rarely—the parasite develops in an epithelium cell belonging to the mucous membrane. This only occurs when the merozoite enters a young epithelial cell lying immediately above the basement membrane but below the nuclei of the fully-formed columnar cells. As the parasite increases in size, it causes its host-cell to bulge through the basement membrane, which finally gives way; and the cell, with its contained parasite, thus falls into the subepithelial connective tissue[3], where its further development is similar to that described in (1) above. The parasite is, in this case, intra-

[1] The youngest stage figured by Siedlecki (1898, Pl. VII, fig. 1) is such an organism. It is not a merozoite passing through the epithelium, but a young parasite developing in an epithelial cell. I have seen many similar parasites in my own preparations.

[2] The hypertrophy of the nucleus is never so great as that seen in the invaded cells of *Octopus*. Cf. p. 37, *supra*.

[3] A similar method of development has been described in "*Eimeria stiedae*" (really *E. perforans*) by Theobald Smith (1910). In this case, however, the parasite is normally an inhabitant of the intestinal epithelium (of the rabbit), and its passage into the submucous tissue is an aberration.

cellular; but although it lies surrounded by connective-tissue cells, its host-cell is an epithelial cell derived from the mucous membrane.

At later stages of development the capsule of every parasite is reinforced by an inner layer contributed by the organism itself. This will be described later. I mention it here in order to emphasize that the cyst-like structures investing the parasites are neither absent—as some earlier workers believed—nor simple. Yet it appears to be immaterial to the parasite whether it lies inside a cell or outside one, and whether its host-cell—when it has one—belongs to the epithelium or to the connective tissue. So long as the parasite is able to enter the submucous tissue it is able to develop—intercellularly or intracellularly: and although it cannot complete its development in the mucous membrane, this is evidently not because the epithelial cells themselves inhibit its growth, since it can develop inside an epithelial cell transported into the submucous tissue. Growth is doubtless dependent mainly upon food supply, and the parasite gets its nourishment by absorbing the necessary constituents from the lymph in the connective tissue of the cuttlefish. In the columnar epithelium, where there are no lymph-spaces, it probably dies of starvation.

In the youngest parasites from the epithelium, the nucleus, as in the merozoites, has an elongated and coarsely granular structure (Fig. 8). It measures some 4μ in length, and lies more centrally than in the merozoite (in which it is terminal and posterior; cf. Pl. VI, fig. 198). It possesses a very thin limiting membrane, surrounding a number of deeply stainable granules of chromatin. In addition, it contains a somewhat larger and more palely staining oval or rounded body (Figs. 1, 2, 8), usually situated towards one end. This body develops into the karyosome of the adult parasite. It is invisible in the nucleus of the merozoite, but gradually appears among the chromatin granules after the parasite has entered the epithelium. At first it is very small; but it soon becomes larger than any of the chromatin granules, and by its different staining reactions becomes conspicuous (Figs. 1, 2). I can give no other information concerning its origin[1]. Its appearance is consistent with the supposition that it is derived from one of the "chromatin granules" which gradually grows larger than its fellows, and at the same time becomes less chromatic: but that the karyosome really arises in this way, and is not present before it is distinguishable, I cannot state.

When first seen, the karyosome is a solid structure (Fig. 2), and homogeneous. Very soon, however, it begins to grow, with the growth of the parasite as a whole; and it can then be seen that it becomes hollow (Figs. 4, 5, 6, etc.), and consists of a rather thick cortex surrounding a central cavity. As it increases in size, its cortex stains more deeply with chromatin stains;

[1] I may note here, as a curiosity, that Moroff (1908, p. 192) interprets the formation of the karyosome at this stage as "parthenogenesis": but the theoretical considerations which led him to such a conclusion do not seem to me to merit discussion. In an earlier work (Moroff, 1906*a*, p. 73) he stated that the karyosome of the young parasite (no species mentioned) is formed by the union of four or five chromatin granules. No evidence in support of this statement was brought forward in his final publication, however, and it is not applicable to *A. eberthi*.

while simultaneously the surrounding granules of chromatin gradually stain more and more feebly, and finally almost disappear (Figs. 2–7). The appearances suggest that their chromatin passes into the karyosome, though this cannot be directly observed.

There is one granular structure, however, which does not behave in this way. Even at a very early stage (cf. Figs. 1, 8) it can usually be seen that one of the chromatin masses in the nucleus is slightly larger and stains somewhat more intensely than the others. This body, which I shall henceforth call the *micronucleus*—for reasons which will be given later—first becomes visible in the nucleus at the time when the karyosome makes its appearance, and is usually to be seen lying in contact with it (Figs. 1, 8). At first it is not distinguishable from the chromatin granules (as in Fig. 2); but it soon grows and becomes sharply differentiated from them (Figs. 3, 4, 5), and stains more deeply with chromatin stains than any other constituent of the organism. In its earliest stages it is a minute granule, about 0·3 μ in diameter. It may be spherical, but is often somewhat irregular in form, appearing oval, rod-shaped, or pear-shaped (cf. Figs. 1, 3, 4, 5, 8); and it generally lies peripherally, in contact with the nuclear membrane (Figs. 3, 8). When the peripheral chromatin granules disappear, this body persists, and gradually grows larger and more distinct.

In their earliest stages of growth the parasites increase in width more rapidly than in length (Figs. 1–6), and their nuclei change from an elongate to a spherical form. The karyosome—now a hollow vesicle—soon begins to show a further differentiation. At one point in its cortex it becomes perforated by a minute pore—the *micropyle*, as I shall call it[1]: and it is directly over or upon this opening that the micronucleus takes up its position. Young parasites at this stage have the characteristic appearance shown in Fig. 5. They have a broader form than a merozoite, but still appear more pointed at the anterior than at the posterior end. The nucleus is now spherical and central, and measures about 2·5 μ in diameter. Its membrane is thin, and the peripherally disposed chromatin granules, which lie in a narrow zone beneath it, now stain but feebly. The most conspicuous structure is the central hollow karyosome, measuring some 1·5 μ in diameter, with its micropyle apparently plugged by the overlying minute but densely stained micronucleus.

The appearances just described are not those which are visible in an occasional parasite, or products of some irregularity of technique. The micronucleus is present in every young parasite, and can be demonstrated by all good cytological methods. It is a constant morphological element of every nucleus at this stage, and with adequate magnification and appropriate staining is, indeed, the most conspicuous structure in the whole organism[2].

[1] This aperture was first observed by Schneider (1883), who called it the "canal micropylaire du nucléole." It was also accurately noted by Siedlecki (1898), though he merely refers to it as "une petite ouverture dans le paroi du karyosome."

[2] It is perhaps hardly necessary to point out that, as the micropyle and micronucleus may lie at any point on the surface of the karyosome, they do not always appear under the microscope

Exceptionally, two micronuclei are present; and when this is so, one of them always lies over the micropyle, in the usual position, while the other lies a short distance from it but in contact with the nuclear membrane (Fig. 7). Not uncommonly, when two micronuclei are present they are united by a slender thread (Fig. 6). Such appearances are not rare in very young parasites, but are very seldom seen in older ones. In these, the micronucleus sometimes appears as a bilobed body or dumb-bell: and it therefore seems probable that partially separated micronuclei (such as are shown in Figs. 6 and 9) may reunite into a single body at a later stage. Very rarely, however, they separate completely, and the two separate micronuclei behave as independent individuals in subsequent development[1].

Only one other change occurs in the young parasite's nucleus before it begins to show definite signs of sexual differentiation. This is the complete disappearance of the peripheral chromatin, so that the organism acquires the simple nuclear structure shown in Fig. 12. Its nucleus is approximately spherical and bounded by a delicate membrane. At its centre lies the single large hollow karyosome, with a single micronucleus lying like a plug over its micropyle. The space between the karyosome and the membrane is now entirely free from chromatin. It is filled with a homogeneous substance (nuclear sap) which shows no structure in the living organism, but appears more or less reticulate or alveolate in fixed and stained specimens. This part of the nucleus is readily stained with "plasma stains"—only the karyosome and micronucleus staining with "chromatin stains." (For example, a nucleus at the stage shown in Fig. 12, when stained with safranin and light green shows karyosome and micronucleus red, the rest green: or stained with Mann's stain, karyosome and micronucleus red or reddish purple, rest of nucleus sky-blue: and so on.)

The micronucleus of the undifferentiated parasite was first accurately observed by Siedlecki, who called it the "secondary karyosome" and believed that it "plays the part of an intermediary between the chromatin of the peripheral nuclear network and that forming the cortical layer of the primary karyosome."[2] He described and figured it[3] up to the stage when it lies over the micropyle, and rightly emphasized the fact that its presence is normal[4], and not the result of degeneration: but he appears not to have followed its development beyond this stage. Despite Siedlecki's observations, however, Moroff seems to have overlooked the micronucleus, or to have regarded it as merely an accidental "aggregation of several chromatin granules."[5] At all events he does not depict it properly in any of his figures[6] of the early stages

exactly as they are shown in the figures. Often the micronucleus lies upon or underneath the karyosome, as presented to the observer, and its presence and precise position can only be made out by proper focussing.

[1] The behaviour of such abnormal nuclei will be described later (p. 48).

[2] Siedlecki (1898), p. 810. [3] *Ibid.*, Text-figs. A–D, p. 810, and Pl. VII, figs. 1–3.

[4] *Ibid.*, p. 809. The statement is italicized in the original.

[5] Moroff (1908), p. 93. [6] *Ibid.*, Pl. VIII, figs. 60–66.

of *A. eberthi*. Pixell-Goodrich (1914) and other observers do not mention the micronucleus at all.

(2) *Development of the Female Parasites.*

The youngest forms hitherto described are all alike, but before they have grown much further they become recognizably differentiated into males and females[1]. Each of the former ultimately forms a large number of small microgametes, while each of the latter becomes converted into a single large macrogamete—as in other Coccidia. The earlier nuclear changes are closely similar in parasites of both sexes.

Apart from later nuclear changes, the males and females are chiefly distinguishable from one another by the structure of their cytoplasm. As a general rule, the males have denser and more stainable cytoplasm than the females; and consequently, when fixed and stained, they appear darker and show smaller cytoplasmic alveoli. Both males and females also contain numerous characteristic cytoplasmic inclusions, though the males contain more than the females after most methods of treatment (cf. Pl. II, figs. 35 and 36).

The cytoplasmic inclusions of *Aggregata* are so varied that they deserve study by themselves, and I shall not attempt to describe them here in detail. Almost every method of fixation and staining produces a different cytoplasmic picture, and many of these pictures are difficult to correlate with one another. Although I have spent much time in studying these inclusions, I am still unable to give a complete account of them all. To do so it would be necessary to study still more material, fixed and stained specially for the purpose. (Most of my material was preserved for the special purpose of studying the nuclear structures.) I shall only note here, therefore, that the cytoplasm—of both sexes—may contain, at various times, the following inclusions in greater or less abundance: (1) granules or small lumps of a material which is closely similar to, or identical with, chromatin; (2) granules of a similar substance, which is probably volutin; (3) droplets of free fat, and probably other fatty or lipoid substances; (4) glycogen and paraglycogen; (5) rod-like, filamentar, or rounded structures of variable size, which resemble the chondriosomes (mitochondria) of metazoan cells[2]. I have studied these inclusions in some detail, but am still in doubt as to their correct interpretation; for they may be demonstrated by methods which are not suitable for staining the mitochondria of metazoa, and they show various peculiarities in their behaviour.

[1] These are usually called "microgametocytes" and "macrogametocytes"—in my opinion cumbrous and unnecessary words which are exactly synonymous with "male" and "female" respectively (in all other organisms).

[2] To this list should perhaps be added a "Golgi apparatus." This has recently been described in *A. eberthi* by Joyet-Lavergne (1924). I have not studied this myself, and regard these structures (or precipitates?) with misgivings. I am not convinced that all things called a "Golgi apparatus" are the same, and the methods used for their demonstration (or production) are often well calculated to produce artifacts. See also Joyet-Lavergne (1924 *a*).

(They are usually well shown in male parasites fixed in formol-sublimate and stained with alcoholic iron-haematein, cf. Pl. II, fig. 36. But the larger inclusions of this sort are also well shown by many other methods of fixation and staining; cf. Pl. II, fig. 38.) I have not attempted to show all the cytoplasmic structures in detail in the figures illustrating this memoir.

Differences in the cytoplasmic structure of males and females are always recognizable, whatever methods of fixation and staining may have been employed. Figs. 35 and 36 (Pl. II) may be taken in illustration. These two parasites—female and male, respectively—were treated exactly alike in every way throughout. The figures are drawn from two individuals, at approximately the same stage in development, lying side by side in the same section of the caecum of a *Sepia*.

Every parasite possesses a well-developed pellicle, or limiting membrane, which can be easily demonstrated in sections. Siedlecki's statement (1898) that no such investment is present is certainly incorrect, as Léger and Duboscq (1908) and others have already pointed out.

We left the young (undifferentiated) parasite with its nucleus in the stage shown in Fig. 12 (Pl. I). Even at this stage the micronucleus, under a high magnification, no longer appears homogeneous, but has a slightly vesicular structure. In this state, and while still very small, it enters the micropyle of the karyosome. This entry is shown in Fig. 13, which represents the nucleus of a larger parasite already definitely recognizable as a young female. As the micropyle is smaller than the micronucleus, the latter is always squeezed out of shape in passing through. A small bit of it goes through first, soon followed by the rest. Midway—as in Fig. 13—the micronucleus appears as a minute vesicular dumb-bell. The end which has gained the interior then enlarges, leaving only a short stalk lodged in the micropyle and projecting through it (Fig. 14). That part of the micronucleus which has entered the karyosome soon acquires a very definite structure. It appears as a minute vesicle showing a distinct chromatin reticulum, but is often slightly irregular in shape. The micronucleus remains in this position—as in Fig. 14—for a considerable time, and grows concomitantly with the rest of the organism.

I would here emphasize the fact that the phenomenon just described is not an arbitrary interpretation of certain unusual appearances. I have examined hundreds of parasites, excellently fixed and stained, at every stage, and they all tell the same story. How the micronucleus is able to effect an entry in this way I do not pretend to understand or explain: but that it does so enter there can be no doubt. It is impossible to misinterpret the appearances. Moreover, as we shall see presently, exactly the same process occurs in male parasites also, and in young schizonts. In every young parasite, therefore, whether it be sexual or asexual, a micronucleus is formed and passes through the micropyle into the karyosome, where it grows into a vesicle.

It has been noted already that very rarely there are two separate micronuclei in the young undifferentiated parasites. When this is so, they both

enter the karyosome at the same time but through separate micropyles[1]—the karyosome, in such individuals, possessing two of these pores—and when inside they both enlarge exactly as in the case just described where only one is present. Such doubly micronucleate individuals are, I believe, abnormal, but I have not succeeded in tracing their subsequent development satisfactorily. The later stages are extremely rare: and for this reason I have sometimes thought that such individuals often do not develop further, and finally die. Yet occasionally I have found specimens which appear to indicate that they may survive, but that only one micronucleus becomes functional—the other degenerating at a later stage. In these rare individuals the normal nuclear changes (which will presently be described) are passed through in the usual way; but during them a second micronucleus can be seen lying unaltered inside the karyosome—just as it does in the early stages. Such an "accessory micronucleus" may occasionally persist up to the time of fertilization (in the female) or the first spindle (in the male). It is also possible that in some cases one of the two micronuclei degenerates at an early age, leaving the other to function alone in the ordinary manner. I have seen several specimens which support this interpretation. Finally, it is possible that all these different methods of development may occur in different individuals; and this is my belief at present. It is certain, in any case, that two micronuclei are occasionally formed, and when this is so they both enter the karyosome: and it is almost certain that in such cases, if the parasite survives, only one of them takes part in the subsequent nuclear development—the other ultimately degenerating, though the stage at which it does so is probably variable.

The young female parasite (Fig. 14) grows during and after the entrance of the micronucleus into its karyosome. It finally becomes a large oval or almost spherical organism possessing an enormous nucleus with a very large karyosome; but during these growth stages nuclear changes also take place—which will be described presently. These changes are connected, in part, with the transformation of the female parasite into a macrogamete, and it is not possible to determine the exact point at which this transformation begins, except by reference to the nuclear changes; for growth continues at the same time as differentiation, and the size of the individual varies within wide limits at every stage. The size ultimately attained appears to depend mainly upon the environment. In the caecum, where the parasites are usually crowded, and accommodation is limited, a "full-grown female" parasite—*i.e.*, before conversion into a macrogamete—may measure no more than about 30μ in diameter: but similar forms in the intestine, where the infection is usually sparser, and growth unimpeded, may reach a diameter of 170μ or even more. (The gametes formed from such specimens are larger in each case.) Except for their size, all these females are closely alike in every respect.

I have made a large number of measurements of parasites at all ages, but

[1] The same phenomenon has been observed in the young schizonts by Léger and Duboscq (1908), and will be referred to in this connexion later (p. 101).

they supply no other information concerning growth. I will give here, however, in support of my general statements, a sample of the measurements made (Table V). This Table records measurements of 300 female parasites from the caecum and the intestine (150 from each). They were, in each case, made from a single fixed and stained film (*i.e.*, from whole parasites). Only well-fixed and undistorted specimens were measured, and only those which showed definite differentiation as females but which had not undergone conversion into macrogametes. (The distinction is often difficult to draw in both cases, but the difficulty was surmounted by rejecting all doubtful individuals.) Almost all these forms were ovoid or elliptical in outline, very few being truly circular. The measurements here recorded represent the greatest diameter, and all were made with an ocular micrometer and are given to the nearest whole division on the scale. (Each unit represents 3·25 μ.)

Table V.

Measurements of 300 *female individuals of* A. eberthi (*fixed and stained*) *from* Sepia officinalis.

Diameter (in units of ocular micrometer)	No. of individuals in		Diameter (in units of ocular micrometer)	No. of individuals in	
	Caecum	Intestine		Caecum	Intestine
7	2	0	30	0	9
8	5	0	31	0	2
9	6	0	32	0	1
10	8	1	33	0	4
11	9	2	34	0	0
12	17	3	35	0	2
13	8	2	36	0	3
14	7	4	37	0	1
15	10	9	38	0	2
16	7	10	39	0	0
17	15	12	40	0	6
18	16	9	41	0	0
19	4	8	42	0	0
20	12	12	43	0	0
21	7	4	44	0	1
22	3	2	45	0	1
23	11	8	46	0	0
24	0	4	47	0	0
25	3	10	48	0	0
26	0	3	49	0	0
27	0	4	50	0	0
28	0	6	51	0	1
29	0	2	52	0	2
				150	150

Converting the figures given in Table V into microns, and summarizing and averaging the results, we reach the conclusions shown in Table VI.

Table VI.

Measurements of 300 *females of* A. eberthi (*whole, fixed and stained*): 150 *individuals from each site.*

Site	Mean Diameter	Extreme Range
Caecum	51·0μ	22·75μ– 81·25μ
Intestine	76·7μ	32·50μ–169·00μ

It should be mentioned that the films from which these measurements were made were not treated in the same way. The caecal parasites were fixed with Flemming's fluid and stained with safranin, whereas the intestinal ones were fixed with Schaudinn's fluid and stained with Delafield's haematoxylin. This difference in cytological method, however, is not sufficient to account for the differences in size, which are equally easily observable in living specimens.

Female parasites, at stages later than that shown in Fig. 14, may contain one karyosome or more—any number up to about 20 (rarely more). But in every individual there is always one main karyosome—that previously called "*the* karyosome"—which is distinguishable by its greater size and peculiar structure. The others are daughter-karyosomes produced by its "budding," and they may at times almost equal the main or "mother" karyosome, though they are usually of various smaller sizes. (Cf. Figs. 16, 19, 24 and 25—the latter being later stages, but showing an arrangement of karyosomes frequently seen much earlier.)

The number of karyosomes appears to be inconstant, and to bear no relation to sexual differentiation and but little to growth or to the size of the parasite. Large organisms may be seen with only one gigantic karyosome (20μ or even more in diameter) whilst small individuals occasionally have ten or more smaller ones. As an example of the results which I obtained when attempting to ascertain whether the number of karyosomes bears any relation to the size of the individual, I give here the numbers actually found in the 150 caecal parasites whose measurements have been recorded in Table V. These are shown in Table VII.

Table VII.

Showing the number of karyosomes present in 150 *females of* A. eberthi (*small caecal parasites*).

Diameter of individual (in divisions of ocular micrometer[1])	No. of individuals	No. of karyosomes in each individual
7	2	1, 1
8	5	1, 1, 1, 1, 1,
9	6	1, 1, 1, 1, 1, 1,
10	8	1, 1, 1, 2, 1, 1, 1, 1
11	9	1, 1, 1, 1, 1, 1, 1, 1, 1
12	17	1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 3, 1, 1, 1, 1
13	8	1, 1, 1, 1, 1, 1, 2, 1
14	7	1, 1, 7, 1, 3, 1, 1
15	10	1, 2, 1, 1, 1, 1, 1, 1, 1, 1
16	7	1, 1, 1, 5, 1, 1, 1
17	15	1, 1, 2, 1, 1, 1, 1, 1, 1, 1, 1, 2, 1, 2, 2
18	16	1, 1, 1, 2, 1, 1, 1, 5, 1, 1, 1, 1, 1, 1, 1, 4
19	4	1, 1, 1, 1
20	12	1, 1, 2, 2, 3, 1, 1, 1, 1, 1, 1, 1
21	7	1, 1, 4, 1, 1, 1, 1
22	3	1, 1, 1
23	11	15, 1, 3, 2, 1, 3, 1, 1, 1, 1, 1
25	3	1, 5, 6

[1] As in Table V, to obtain actual dimensions, in microns, these numbers must be multiplied by 3·25.

These individuals were taken at random, in the order in which they happened to appear in working systematically through the preparation. Karyosomes which were incompletely separated from the main karyosome, or which were in process of division, were seen in several of these specimens, and in each case were recorded as separate individuals. It so happens that in this series no number of karyosomes greater than 15 occurred: but this is merely a matter of chance. The table shows that a single large karyosome is commonly present in females of all sizes, but that multiple karyosomes may be present in small individuals as well as in large. On the average, however, the large tend to have more than the small. If the 150 individuals in the table are divided into two lots, one containing all parasites with diameters from 7 to 15 units (72 individuals), the other all those from 16 to 25 (78 individuals), then we find that 6 in the first lot (= 8·3 per cent.) have more than one karyosome, and 18 in the second lot (= 23 per cent.).

These figures will serve as a specimen. I attach but little importance to the actual numbers found, however, because there is such wide variation in the size of individuals and the number of their karyosomes that I think no satisfactory numerical statement is possible. The only karyosome constantly present at all stages is the main karyosome: while daughter-karyosomes derived from it may be present in all except the earliest stages, but in numbers which are inconstant.

Much has been written by earlier workers—Schneider, Labbé, Mingazzini, and the rest—regarding the formation of the daughter-karyosomes; but there is no unanimity in the matter, and I must confess that I am still in some doubt regarding certain details. It is generally accepted that the daughter-karyosomes are "budded" from the big one; but exactly how this budding is effected is very difficult to determine. The process cannot be observed satisfactorily in living specimens, and stained individuals are not always easy to interpret.

At an early age the main karyosome is a hollow sphere with a thick rind (*e.g.*, Figs. 12–14) which appears homogeneous and stains deeply with chromatin stains. Its central part, or medulla, consists of a denser protoplasm than the surrounding nuclear sap: and though both medulla and sap usually show a definite alveolar structure in sections (cf. Fig. 16), I believe this is a product of fixation. In living specimens, at any rate, the contents of both the nucleus[1] and the karyosome appear homogeneous. Thin sections of the karyosome (Figs. 16, 17) always show a condensation of the medullary protoplasm on the inner surface of the cortex, so that it appears to consist of two strata—a thick, darkly-staining outer layer, and a thin palely-staining inner. But this inner layer I believe to be also an artifact. It is probably merely a denser deposit of medullary protoplasm resulting from fixation.

[1] According to Moroff (1908) the nuclear sap (*i.e.* the substance contained within the nuclear membrane, and in which the karyosome is imbedded) contains vacuoles in certain "species" of *Aggregata*. Such vacuoles are always, in my opinion, artifacts. Normal, well-fixed specimens—of any age or either sex—never show them.

Now if budding were to occur from the cortical layer, one would suppose that figures such as Fig. 15 would be commonly met with in preparations. One would suppose that diverticula would be formed at various points—as in this figure—and then be constricted off as "buds"; and such buds would thus be hollow, like the original karyosome. But the fact is that, although daughter-karyosomes are very frequently formed and may often be seen attached to the main karyosome, specimens such as Fig. 15 are excessively rare in preparations; and moreover, the daughter-karyosomes, when first formed, are hardly ever hollow[1]. They often appear denser at the periphery, and somewhat spongy in the middle (as in the largest daughter-karyosome shown in Fig. 19), but this appearance is to be expected in any homogeneous drop of colloidal substance after fixation. I do not believe that the karyosome in Fig. 15 is budding, but that it is abnormally distorted. It is possible that buds may be formed in this manner in very young parasites, in which the cortex is comparatively thin and soft; but in larger individuals, with a thick hard cortex to the karyosome, I am confident that they are not.

Moreover, the daughter-karyosomes are usually formed in the region of the micropyle, around which clusters of all sizes may often be seen (cf. Fig. 19). Very often, too, these karyosomes are united to the micropyle by stalks of variable length and thickness. The appearances strongly suggest that they are formed from the medullary protoplasm, which is extruded, like a drop of excretion, through the micropyle, and finally constricted off. I believe that most of the daughter-karyosomes are formed in this manner, though it is difficult to prove that this is so. Usually the micronucleus lies just inside the micropyle (as in Fig. 19), which it appears to plug more or less effectively: but at times it lies at some distance from the micropyle, though it then generally sends a long process or outgrowth into it. This process usually terminates in a bead-like plug externally (Fig. 20). Such a condition is easily derivable from that shown in Figs. 13, 14, and 15. Notwithstanding that the micropyle is thus plugged by the micronucleus, it is probable that the contents of the karyosome can escape round the plug. Figures such as Fig. 19 certainly suggest that it does so; and if it does not, I am at a loss to explain the origin of the secondary karyosomes.

It must be noted, however, that small karyosomes are sometimes seen attached to the main karyosome at points remote from the micropyle. These are explicable by the fact that secondary micropyles are often formed in older karyosomes, and buds may therefore be extruded through these. In very thin sections several micropyles, in addition to the original or main micropyle, can sometimes be demonstrated (Fig. 16 shows four). But these do not appear to be constant in number or position, and it is possible that they are temporary formations[2].

[1] They often become definitely differentiated into hollow spheres—showing a distinct cortical layer surrounding a less dense medulla—at a later stage. The cortex, in such karyosomes, shows no micropyles.

[2] It is also possible that they are only cracks produced in the cortex artificially in the process of sectioning: but I think this unlikely.

Daughter-karyosomes, when first formed, stain less deeply than the main karyosome: but as they grow larger, and acquire a definite cortical layer, they stain more intensely, until they may appear as chromatic as their parent. They also divide into two[1] or break up into several smaller karyosomes. Possibly, also, the cortex itself may be able to form and constrict off droplets from its outer surface: but I have found no conclusive evidence of this.

My conception of the origin of the secondary karyosomes appears to agree more closely with that of Schneider than with those of most other observers. Schneider called the main karyosome the "principal nucleolus," and the daughter-karyosomes "secondary nucleoli" or "nucleolites." He came to the conclusion that the former does not divide, but "buds off" secondary karyosomes by a process "so peculiar that the name [*i.e.*, budding] is only half appropriate for it."[2] If I understand him aright, he believed that the buds were formed by an excretion of karyosomic material through the micropyle[3]. Siedlecki[4], however, seems to have interpreted the external knob of the micronucleus (my Figs. 14, 18, 20, etc.) as itself a daughter-karyosome. He did not observe the entry of the micronucleus ("secondary karyosome") through the micropyle, and regarded the knob as a "bud," which increases in size and is finally constricted off as a daughter-karyosome. Further budding then occurs—in his view—in the same way, and the daughter-karyosomes afterwards form similar buds. This account is irreconcilable with my observations.

It is probable that Labbé (1896) confused the disintegration of the karyosome (occurring after fertilization) with the formation of "buds"; but his views on the phenomena in question are so evidently based upon insufficient knowledge of the facts that I shall not discuss them in detail. I may note, however, that his statement[5] that the daughter-karyosomes ("karyosomes primaires") are formed inside the main karyosome ("karyosome initial") and "from its inner walls," is indubitably wrong.

Whilst the budding of the karyosome has been taking place, the micronucleus has undergone further changes, which have been noted, in part, already. This body, when it has gained the inside of the karyosome, acquires a very definite nuclear structure. Fig. 18 shows the micronucleus, still lodged in the micropyle, in a section through the nucleus of a fairly large female. Fig. 17 shows it lying entirely within the karyosome. Fig. 19 shows another specimen, differently stained. Fig. 20 shows a later nucleus, but illustrates a condition commonly observable at earlier stages—with the micronucleus

[1] Stages in the division of the daughter-karyosomes are fairly common in preparations. The division is effected by constriction, with the formation of the usual dumb-bell shaped figures. I have not illustrated these phenomena—for lack of space.

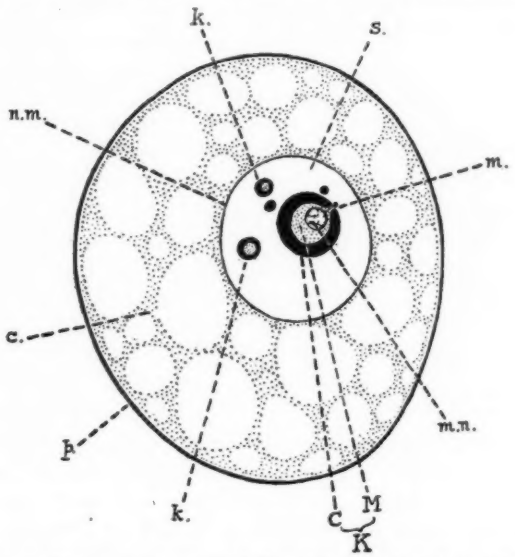
[2] Schneider (1883), p. 84.

[3] Schneider's observations appear to have been made chiefly on an *Aggregata* from *Octopus* (*A. octopiana*?), in which the conditions—according to my observations—are probably a little different from those which I have observed in *A. eberthi*.

[4] Siedlecki (1898), pp. 812, 813.

[5] Labbé (1896), p. 576.

drawn out into a process which fits into the micropyle and is surmounted by a knob or plug. (At earlier stages this process may be a mere strand, as in Fig. 15.) In all these figures, and in all good preparations of these stages, the micronucleus has the same structure. It is a small vesicle, showing a definite membrane and a reticular arrangement of chromatin. In size it is proportional to the karyosome in which it lies (see the figures). It is present in every nucleus, though it is not, of course, visible in every section through a nucleus (cf. Figs. 16, 17). Owing to its delicacy, to the difficulty of studying whole preparations after staining, and of cutting perfect serial sections of large



Text-fig. 2. Diagrammatic representation of the structure of *A. eberthi* (♀).

karyosomes[1], it is not always easy to demonstrate its presence. In imperfectly fixed and stained specimens, and in large whole parasites, it can, indeed, rarely be made out; but this is only to be expected, and can hardly be taken as evidence of its absence on such occasions.

I may now summarize what has just been said about the nuclear structure of the female parasite (see Text-fig. 2). The "nucleus" (*i.e.*, the structure to which this name has previously been given) is a large rounded or oval vesicle furnished with a definite membrane (*n.m.*) and lying in the cytoplasm (*c.*) near the middle of the organism, which is invested with a thin pellicle (*p.*). The

[1] The main karyosome, when fixed, is a large, dense and often very hard body, lying in a much softer matrix. Consequently, it is readily displaced by the knife of the microtome, and sometimes merely chipped or broken instead of being properly sectioned. It is quite common, in sections of 5μ–10μ, to find karyosomes much thicker (10μ–20μ in diameter), which have not been sectioned at all. The knife passes over instead of through the karyosome, leaving it whole in one section, with a hole of corresponding size in the preceding and succeeding sections.

nucleus contains a large, more or less central, main karyosome (*K*), consisting of two parts—a thick rind or cortex (*C*) perforated at one point by a micropyle (*m.*), and a central protoplasmic medulla (*M*) containing a small vesicular structure which I call the micronucleus (*m.n.*). This body usually lies in relation to the micropyle as shown in the figure. The nuclear space (*s.*), between the karyosome and the membrane, is filled with a homogeneous non-chromatic substance, but often contains one or more small accessory karyosomes (*k.k.*) which are formed by "budding" from the main karyosome. The budding is probably effected by the extrusion of drops of karyosomic substance (medulla) through the micropyle. All the chromatin of this nuclear system is contained in the karyosomes and the micronucleus.

(3) *Formation of the Macrogametes.*

As already noted, the female parasites develop into macrogametes—each female becoming converted into a single large gamete. The process of conversion is long and complex, and not at all the simple affair[1] that one might suppose it to be from the published accounts of *Aggregata* and other Coccidia.

The first noticeable change occurs in the nucleus. In females which are about to become macrogametes, minute granules or droplets of faintly stainable material begin to emerge from the main karyosome (Figs. 19, 20). Most of them appear to come through the micropyle, around which they are at first collected (Fig. 20). They soon become more numerous, and arrange themselves in faint threads or chains or little clouds (Figs. 20, 21, 22). Finally the whole nuclear space is filled with threads and granules, which eventually dispose themselves in a rather indefinite network (Fig. 24). All stages in this process are commonly found in preparations, wherefore I conclude that it occurs slowly.

Exactly how this network is formed is difficult to ascertain; but it seems clear that it is derived from two distinct sources. The substance (? linin) which is first "sweated out," as it were, from the karyosome, is probably derived from the medullary material of the karyosome itself. Most of it comes through the micropyle, but a part may also emerge at other points—probably through the accessory (temporary?) micropyles already mentioned. (Cf. Fig. 21, which shows an appearance suggesting that material is leaving the karyosome at several points.) This substance, which I hesitate to name, is soon supplemented by another material, in the form of minute but more deeply stainable granules or droplets. These are derived from the substance of the micronucleus, and may therefore, I think, correctly be called "chromatin." The micronucleus undergoes complete fragmentation *in situ*, and the whole of its substance is discharged through the micropyle into the nuclear space (Figs. 21, 22).

During these stages the central region of the karyosome presents appearances which vary greatly according to the staining methods used. If haema-

[1] Cf. Siedlecki (1898), p. 822: "La formation de l'élément femelle est des plus simples."

toxylin (Delafield's, iron-, or copper-haematoxylin, etc.) be employed, the whole of the medulla has a dark, granular appearance (Fig. 22), suggesting that it is filled with chromatin. But with many other stains (of which carmine or safranin—as in Figs. 20, 21—are examples) the medulla stains feebly, suggesting that it contains but little chromatin. I am not certain how to interpret these different pictures: but it is clear, from morphological evidence, that the micronucleus breaks up completely and discharges the greater part of its substance into the cavity of the karyosome; and that the material of which it was composed is then excreted through the micropyles into the nuclear space.

In any case, it is certain that the micronucleus ultimately disappears, and numerous stages in its disintegration and disappearance can be demonstrated. For example, in Fig. 22 it is still recognizable, whereas in Fig. 21 only a few granules of chromatin, lying in the position which it occupied, are visible. The specimen shown in Fig. 23 is one of several similar ones which I have been able to find. This figure depicts a very thin tangential section, of the karyosome only, of a very large parasite. The section contains the whole of the micronucleus, which is flattened against the cortex. It shows the micronucleus breaking up, and its substance passing in the form of minute granules through the cortex and into the nuclear space. Fig. 27 shows the karyosome of a parasite at a slightly later stage—from a section stained with paracarmine. The micronucleus has vanished, but chromatin derived from it can here be seen passing through the substance of the cortex, as though it were being excreted.

After the micronucleus has been discharged from the karyosome, a few globular bodies can usually be seen lying in the medulla (Figs. 21, 27). These bodies, which I call the residual granules of the karyosome, are of variable size, slightly refractile, and stain very feebly with most stains. They often lie irregularly scattered through the medulla, but often also they form a short chain—especially at later stages—and appear to be passing out in single file through the micropyle (Fig. 27). As a rule they do not all pass out, and a few can often be seen even after fertilization has taken place. Whether they are derived from the micronucleus or from the karyosome itself I have been unable to determine.

During the disintegration of the micronucleus—or even earlier—the cortex of the karyosome generally undergoes a change. It acquires a spongy appearance, becomes more or less vacuolate, and often develops bright granular inclusions in its substance—the granules varying greatly in number. Figs. 20, 21, 22, and 27 illustrate these changes, which should probably be interpreted as signs of the degeneration which the karyosome henceforth undergoes.

Whatever may be the correct interpretation of the appearances just described, one thing is, I think, certain: the micronucleus breaks down, and discharges its substance into the nuclear space. This breaking down can be seen in well fixed and stained specimens, and is not the result of faulty

technique; for at all the later stages no micronucleus is present in the karyosome, although in the earlier ones it can always be demonstrated. The nuclear network (Fig. 24)—or at least that part of it which consists of chromatin—is almost certainly derived from the micronucleus, and not from the karyosome.

The fine indefinite network shown in Fig. 24 gradually begins to get more definite and to stain more deeply. All stages in this process could easily be figured, if there were space for them. I can show only two—Figs. 25 and 26. The former shows a whole parasite under a moderate magnification, and illustrates the general appearance of an organism at this stage. Fig. 26 shows the nucleus of a similar form, but more highly magnified. In this figure I have tried to draw the network in its entirety—correct in every detail—though I have not succeeded to my complete satisfaction, as the structure is very complicated. As will be seen, the network consists of linin, studded with chromatin granules, and is attached partly to the karyosomes and partly to the nuclear membrane. (Ends which appear free in Figs. 25 and 26 are really so attached.) Such networks are often even more complicated, and their exact disposition is extremely difficult to make out in large parasites. They have no constant form.

This reticulate figure now resolves itself into a spireme. Parts of it first become differentiated into beaded threads, usually looped and twisted, and later the various pieces become continuous. The spiremes so formed are illustrated in Figs. 28 and 29. The former is an attempt to show an entire spireme thread, as seen under a very high magnification. (Although drawn with great care this figure may not be quite accurate towards the centre, where the thread is so tangled that it is impossible to resolve it with certainty. All the loops, however, are accurately shown.) It will be observed that the chromatin granules are widely spaced at this stage. The other figure (Fig. 29) shows the whole nucleus, at a slightly later stage, in a small parasite. The loops at this stage are very definite, and ultimately they can be counted. There are then always six of them, of different sizes, and always attached to the nuclear membrane at their looped ends. Finally they separate from one another (Fig. 30), and lie within the nucleus as six independent filamentar chromosomes (Fig. 31). At this stage the nucleus contains nothing but the karyosomes and chromosomes, lying in the nuclear sap, which appears almost structureless in well-fixed preparations.

The six chromosomes may be clearly studied, in favourable specimens, at this moment. They are rather coarsely beaded single threads—never double—and are always partly adherent to the nuclear membrane. Fig. 31 was drawn with the utmost care[1], and shows every chromosome as accurately as possible down to the last granule. (In the specimen itself the chromosomes are not all visible simultaneously, as they lie at different levels and their course can only be made out by careful focussing.) I have seen a number of similar

[1] The karyosomes, however, are not drawn in detail in this figure.

specimens in my preparations; but this is one of the best, as the chromosomes are here particularly well separated.

Whenever the chromosomes are clearly countable at this stage there are always six, and they are always different from one another. No two are of the same length. There is one very long filament, and one very short one, while the rest are of intermediate lengths. For convenience of description I call these chromosomes *a*, *b*, *c*, *d*, *e*, *f*, in order of magnitude—from *a* the longest to *f* the shortest[1]. This set of six differentiated chromosomes is a haploid group which occurs again and again, in various forms, at every nuclear division save one in the life-cycle of *A. eberthi*.

The events just described appear to be the prophases of a mitotic division. But no division follows. I have searched very carefully for one, and am confident that none occurs[2]. Instead of dividing, the nucleus now passes to the surface of the organism, and becomes elongated into an ovoid or fusiform body with a more or less radial disposition—*i.e.*, with one of its poles directed towards the surface, the other towards the centre. As the nucleus is drawn out the chromosomes take up an axial position in it (Fig. 32). At this stage the two longest chromosomes (*a* and *b*), usually more or less twisted, generally lie alongside one another, extending almost from one pole of the nucleus to the other, while the smaller chromosomes (*c*, *d*, *e*, *f*), lie about them in various positions. This figure is very characteristic, and appears always to occur at this stage. I call it the "pseudospindle" or "prefertilization spindle" figure, because it resembles the so-called "fertilization spindle" which is formed at a much later stage (after fertilization)—with which it has nothing whatever to do, though it has evidently been frequently mistaken for it in *Aggregata* and other Coccidia by previous observers[3].

This "pseudospindle" figure does not long persist, for the chromosomes soon begin to travel towards the outer pole of the nucleus. Fig. 33 shows a stage in this process. (The nucleus is here viewed obliquely. In reality the point on the right where the chromosomes touch the nuclear membrane is very near the surface of the organism, and the nucleus is much more elongated than it appears—the opposite pole lying very deeply in the section.) During their passage to the pole the chromosomes break up into granules or short strings of granules. In Fig. 33 they are not countable: in Fig. 34—a still later stage—they are no longer even recognizable as chromosomes.

When the chromosomes have completely broken up, and their chromatin

[1] The six chromosomes of Fig. 31 are labelled in Fig. 1, *E* (p. 85) of my preliminary note (Dobell and Jameson, 1915), to which the reader may be referred.

[2] Before I was acquainted with the chromosome cycle of *Aggregata* I imagined that a reduction division ought to take place at this point—as previous workers had suggested. I was therefore greatly puzzled at being unable to find later stages, and spent a great deal of time in searching vainly for them.

[3] It is readily distinguishable from the "fertilization spindle" by the fact that it occurs in an *unfertilized* macrogamete (with characteristic cytoplasmic structure and no fertilization membrane) and by the fact that it is composed of individualized chromosomes, which, when countable, are found to be six in number.

has reached the outer pole of the nucleus[1], maturation is complete. The macrogamete is now ready for fertilization, and undergoes no further change until this occurs.

During the process of conversion of the female parasite into a macrogamete, growth has continued, so that mature gametes are always larger than the females from which they are formed. But it is very difficult to give exact figures for their size, since they vary—like the females—within wide limits. In a crowded caecal infection the mature macrogametes may measure less than 50μ in diameter: in a sparse infection in the intestine they may attain a diameter well over 200μ. All intermediates also occur. As a sample of the measurements which I have made I may record the following. In the course of measuring the 300 females whose sizes are shown in Tables V and VI, I met with a small number of mature macrogametes. The sizes of these are shown in Table VIII.

Table VIII.

Mature macrogametes of A. eberthi.

Site	Mean diameter	Extreme range
Caecum	68μ	49·0μ– 81·0μ
Intestine	146μ	123·5μ–162·5μ

Comparison of these figures with those in Table VI will illustrate the sort of variation met with. But the figures vary from preparation to preparation; and while it is true that macrogametes are, on the average, larger than females, it will be noted that in this particular case the largest female seen was actually larger (169μ) than the largest macrogamete (162·5μ). This, however, is merely because, on this occasion, too few macrogametes were studied, and no very large ones were found.

During maturation the degeneration and fragmentation of the karyosomes continue. Consequently, the mature macrogamete has no constant number of karyosomes, but may show one large one or twenty (or more) smaller ones of various sizes. The karyosomes, or their derivatives, play no part in the maturation phenomena.

Before describing the process of fertilization I must give an account of the development of the male parasites and the microgametes. It will therefore be useful, perhaps, if I here summarize very briefly what has just been said about the maturation of the macrogamete. Each female individual is converted into a single macrogamete as a result of growth accompanied by the following nuclear changes: (1) disappearance of the micronucleus, and the distribution of its substance through the nuclear space; (2) formation of a delicate network—filling the whole nuclear space—from this micronuclear substance; (3) condensation and organization of the network into a continuous looped spireme; (4) separation of the loops into six filamentar chromosomes,

[1] I may add that at this pole, where the chromosomes congregate and break down, there is no centrosome or similar structure. I have searched very carefully for any such body which might be present at this stage, but always in vain.

differentiated individually by their lengths; (5) elongation of the nucleus, with an axial distribution of the six chromosomes to form a "pseudospindle" figure; (6) passage of the chromosomes to the outer pole of the nucleus; (7) breaking up of the chromosomes into an irregular mass of chromatin granules at this spot; and throughout all these stages (8) degeneration and fragmentation of the karyosome.

All the foregoing changes occur, I believe, during the maturation of macrogametes of all sizes, but in large individuals they are much more difficult to follow than in small ones. In all, the chromosomes are alike in form and number—whenever it is possible to count them with certainty. It must be noted, however, that I have made a large number of uncertain counts in my attempts to enumerate them in reconstructions from sections—especially of large individuals; but the technical difficulties involved are amply sufficient to account for errors in such cases, without resorting to the supposition that the chromosomes themselves vary in number in different individuals. There is no good evidence that this is so: on the contrary, all the unequivocal evidence points clearly to the conclusion that the macrogamete, whatever its size, contains a haploid group of six differentiated chromosomes, which are constant in number and form.

As all previous workers appear to have overlooked or misunderstood all the stages of maturation in the nucleus of the macrogamete, there are no earlier findings which require discussion at this point.

(4) *Development of the Male Parasites, and the Nuclear Divisions preceding Microgametogenesis.*

It has already been noted that male parasites soon become recognizable by their cytoplasmic structure and staining reactions. They stain more deeply than females, and their cytoplasm appears denser, more chromatic, and contains more numerous inclusions (when fixed and stained by most methods). Fig. 36 (Pl. II) shows a young male alongside of a young female (Fig. 35) of about the same age, and conveys a good idea of the differences observable between them.

In the earlier stages of development the males pass through the same series of nuclear changes as have already been described in the females. At a very early age the micronucleus lies in the micropyle of the karyosome (Fig. 37): but later it passes through—just as it does in the female—and enters the karyosome, where it enlarges into a definite vesicle showing a typical nuclear structure (Fig. 38). At this stage, therefore, the young male parasite is organized—as regards its nuclear apparatus—exactly like a female[1]. All the chromatin is contained in the karyosome and the micronucleus. The nuclear membrane is well developed, and the nuclear sap is clear, homogeneous, and free from chromatin.

[1] That the organism depicted in Fig. 38 is not a female is shown by its cytoplasmic structure. In other respects Text-fig. 2 (p. 55) would illustrate a parasite of either sex, and may therefore be referred to again here.

Everything that has already been said about the structure of the karyosome in the female applies equally to the male, and therefore need not be repeated. "Budding" of the karyosome occurs in the same way also, and the number of "buds" formed varies in the same irregular manner[1]. Males also vary in size, just like females, according to their site in the host—those in the caecum being small, those in the intestine large. The only difference of note is that males are, on the whole, considerably smaller than females: but it is very difficult to give any precise figures for their dimensions, because they also vary within wide limits. A very small male, in a crowded caecal infection, may measure no more than 20μ in diameter when mature; while a very large male, lying well isolated in the lower intestine, may attain a diameter of over 100μ. All intermediate sizes are fairly common.

The early nuclear changes leading to the formation of chromosomes also occur in the males precisely as they do in females. A nuclear network is formed, apparently from the substance of the micronucleus, in the nuclear space. It is faint at first, but gradually becomes more definite. I figure only one stage, in a small male (Fig. 39), out of a very large number which I have seen. (All stages are common in preparations, but exactly like those already figured in females.) From the network a spireme is formed (Fig. 40), in the same way; and in the same way it opens out into a series of loops—six in number, and unequal in size—which are first attached by their looped ends to the nuclear membrane (Fig. 41) and then break apart as free filamentar chromosomes (Fig. 42). At this stage the chromosomes are sometimes very clear. They are always single threads, always six in number, and differentiated exactly as they are in the female (cf. Pl. I, fig. 31); that is, they form a typical complex *a*, *b*, *c*, *d*, *e*, *f*—from *a* the longest to *f* the shortest. Fig. 42 shows every member of the group clearly, and will require no further explanation[2].

Up to this point the development of the nucleus in the male is exactly comparable with that in the female: but from now onwards it is different. As already noted the changes leading to the formation of chromosomes in the female appear to be—but are not—the prophases of a mitotic division: in the male, on the other hand, they not only appear to be but are; for the nucleus of the male now proceeds to divide by a very peculiar and complex "polymitosis."

When the chromosomes are fully formed (Fig. 42) a small achromatic body becomes visible in the cytoplasm at one pole of the nucleus. This is the rudiment of the achromatic spindle, which is now about to form for the first mitotic division. It soon enlarges and becomes elongate or oval, and finally spindle-shaped. It is shown at this stage in Fig. 43, which also illustrates the behaviour of the chromosomes at this time: for as soon as the achromatic

[1] Secondary karyosomes are usually less numerous in males than in females of *A. eberthi*: but Moroff's statement (1908, p. 106) that no "budding" of the karyosome occurs at all in the male is undoubtedly incorrect (cf. Pl. II, fig. 36).

[2] The chromosomes in this nucleus are traced out and labelled in Fig. 1, *A* (p. 85) of my preliminary note (Dobell and Jameson, 1915).

body has become conspicuous, they begin to stream towards it—as though one end of each filament were attracted to it[1].

I have not given a series of figures illustrating the formation of this achromatic body in its earliest stages because I am unable to do so. It would be easy to figure, from my preparations, a large number of probable stages, but they would all be more or less uncertain. Male parasites usually have numerous cytoplasmic inclusions—as already noted—and these often lie on or near the nuclear membrane at all stages (cf. Fig. 38): and I have been unable to distinguish the spindle-rudiment from these, with certainty, at the time of its first appearance. When it becomes larger—*e.g.*, Fig. 43—it is readily distinguishable with most methods of staining, and its development can be followed with comparative ease afterwards. As regards its origin, however, all I can say with certainty is that, when first recognizable, it always lies on or very near the nuclear membrane, but always outside it—in the cytoplasm, never within the nucleus. That it is originally formed within the nucleus and extruded, I do not believe; but I have been unable to ascertain its origin with absolute certainty[2].

During the foregoing stages the nucleus as a whole has gradually passed towards the periphery of the organism. It finally comes to lie immediately below the surface at one point—the point where the achromatic spindle is forming—and the chromosomes then approach the middle of the spindle and attach themselves to it by their ends (Fig. 44). At this time they are still filamentar, but so close together that they are difficult or impossible to count. But they now shorten and thicken, and ultimately pass on to the equatorial plate as dense, almost spherical masses. At the same time the spindle-rudiment elongates, acquires a fibrous structure, and the typical form of a mitotic spindle. Simultaneously the nuclear membrane disappears, though the area which it enclosed remains recognizable long afterwards owing to its homogeneous appearance.

As the chromosomes shorten and thicken, they can be clearly counted again. At first they assume a variety of peculiar shapes—often appearing clubbed or swollen at the ends—but they finally become more or less spherical (see Figs. 45, 46, 47). The six individual chromosomes are clearly distinguishable throughout, in spite of their altered forms, by their sizes. No two are of the same size, but there is a regular gradation from the largest (*a*) to the smallest (*f*). The figures will illustrate this better than words. I attach importance to all these stages because the counts are here particularly certain. Six differentiated filamentar chromosomes (*a–f*) are formed in the prophases

[1] Males at this stage may be mistaken for fertilized macrogametes showing a "fertilization spindle," or for macrogametes at the stage of the "pseudospindle" (Fig. 32). The spindle rudiment and the cytoplasmic structure afford a key to their identity.

[2] Pixell-Goodrich (1914, p. 164) says that "a centrosome with distinct astral radiations apparently emerges from the nucleus at the beginning of the process, and undergoes repeated division before the nucleus becomes subdivided." No figures of the emergent centrosome are given, and nothing of the sort is apparent in any of my preparations.

(Fig. 42), and six globular but otherwise similarly individualized chromosomes are present on the equatorial plate subsequently (Fig. 47). The same haploid group is present throughout, and no member of the group shows any sign of bivalency.

The six globular chromosomes on the equatorial plate (Fig. 47) now divide at metaphase by simple constriction into two (Fig. 48). Two groups, of six homologous chromosomes each, are thus formed at the early anaphases (Fig. 49[1]). I have been able to observe these stages, in some specimens, with certainty. But as the ends of the spindle draw apart, the chromosomes lose their globular form, become drawn out into irregular filaments along the spindle-fibres, and rapidly become uncountable (Figs. 50, 52).

Whilst the mitotic spindle has been forming, the parasite has undergone another change. Originally, every male—like every female—is closely invested with a delicate but definite membrane or pellicle (cf. Figs. 35, 36). When the spindle is forming, however, this pellicle separates from the protoplasm—in much the same way as the "fertilization membrane" does (as will be described later). The parasite appears to cast its skin, and the space between the "skin" and the protoplasm is then filled with a homogeneous liquid. This space ultimately becomes extensive, and the "skin" itself finally fuses with the cellular investment with which—as already noted—every parasite is surrounded. In this way the male parasite becomes a naked[2] ball of protoplasm lying freely in a capsule, and it remains in this state up to the end of its development. In stained preparations the capsule is often much folded or creased (as shown in section in Fig. 49), but in the living organism it is usually spherical, and appears distended by its contained liquid[3].

From the time of its first appearance as an "achromatic body" (Fig. 43) the spindle seems to be a homogeneous solid structure. As it becomes more pointed at its poles it shows a faint longitudinal striation, but astral radiations —even in the later stages—are only seldom distinctly visible, and then, as a rule, only in large parasites. I have been unable to demonstrate any centrosomes or centrioles with certainty at any stage in well fixed and stained preparations.

In the early anaphases (Figs. 49, 50) the conical ends of the spindle draw apart; and as they do so, they point towards the surface, so that the spindle appears to break in the middle (Fig. 50). As the chromosomes lengthen into beaded filaments, they gradually form a system of branched and irregular

[1] The spindle in Fig. 49 is really lying superficially, in its usual position; but the specimen is viewed from the pole at which it is situated.

[2] I think it must have been from observing males in these later stages of development, and fertilized macrogametes, that Siedlecki (1898) was led to deny that *A. eberthi* is clothed with a membrane in the earlier stages.

[3] The size of the space intervening between the protoplasm and the capsule varies greatly, and depends chiefly upon the amount of room available to the parasite in the tissues. In a crowded infection, the capsule may invest the parasite fairly closely, even at much later stages (as in Figs. 51 and 56), while in sparse infections (as in Fig. 49) it may expand to large dimensions at a very early stage.

loops—apparently connected together—which sink towards the centre of the organism. Simultaneously the achromatic cones at the spindle ends become shorter, and come to lie immediately below the surface. The nucleus thus acquires the characteristic appearance shown in Fig. 51. (Fig. 52 shows a surface view of a similar but larger "spindle" at a slightly earlier stage.)

These and the ensuing figures are excessively difficult to study. I am convinced, however, that they are formed in a fairly simple manner. At the stage shown in Fig. 50 the chromosomes grow out into long filaments, which a little later become continuous along the spindle-fibres. A series of beaded chromatin threads is thus formed, extending almost from pole to pole and uniting the two anaphase groups into a continuous system. Whether continuity is thus established between homologous chromosomes I have been unable to determine, but I think it is very probable. As the threads grow longer, they split longitudinally but incompletely, and so form the network seen in Fig. 51. It will be observed that the polar end of each chromosome becomes recurved or hooked, and that the loop becomes attached to the achromatic cone while the extremity remains free and directed outwards (Figs. 50, 52, 51).

The figure now becomes much more complicated. The achromatic cones form small spindles—like the original spindle, but smaller—and draw apart. The chromosomes attached to them split completely, and one daughter-group follows each daughter cone (Figs. 53, 54). Four chromosome-groups thus result. But the same process is now repeated a number of times—probably rapidly—and a most complicated figure results (Fig. 56). I call this stage the "polyaster stage." It is always possible to distinguish a variable number of "asters"—each formed by an achromatic cone with its attached chromosomes—lying at the surface; whilst the centrally directed ends of the chromosomes form an inextricably tangled maze of threads in the middle of the organism. The tangle results from repeated but only partial splitting of the chromosome threads (cf. Fig. 55). They split and open out, but remain attached: then split again, more or less completely, and again open out into irregular loops: and so finally produce an irregular net. Fig. 56 is drawn from a specially selected very small male, which is represented as accurately as possible. (In reality the polyaster is even more complex, because several other "asters" are present—above and below the central tangle, but all lying on the surface of the organism—and it was impossible to draw these without obscuring the rest of the figure.) In large males, in which over 20 asters may finally be formed, the conditions are precisely similar but much more complicated. Such large specimens are extremely difficult to study, and to reconstruct the whole polyaster system accurately from serial sections is usually impossible. I have therefore figured, as well as I am able, the simplest cases only. From these the constitution of larger and more complex individuals can, I think, be readily understood.

The number of "asters" resulting from this polymitosis is variable, but

depends in part upon the size of the original organism. Very small males may form only six, eight, or ten: larger ones more than 20. Odd numbers are often formed—*e.g.* seven, eleven, etc.—because the asters do not usually divide synchronously, especially in the later stages. After a time, however, the divisions cease, and the asters become uniformly spaced on the surface of the organism (Fig. 57). They then begin to extricate their chromosomes from the central tangle. Each aster ultimately emerges and becomes free: and it is then possible, for the first time, to see that it is composed of a small achromatic cone with a set of six filamentar chromosomes attached to it (Fig. 58). In many of these emergent groups the six chromosomes can be clearly counted, and every individual (*a–f*) can be recognized. (Five of the ten groups in Fig. 58 show the typical haploid complex clearly.) As soon as they have emerged, the chromosomes appear to roll themselves into loose balls of thread (four of the groups are in these stages in Fig. 58), and then become transformed into resting nuclei (Fig. 59) at the surface of the organism. The resting nuclei so formed are delicate vesicular structures, and always stain—after the chromosomes have disappeared during the telophases—very feebly. The achromatic cones persist, and can be seen as minute nipples, lying outside the nuclear membrane and projecting slightly above the surface. This state of rest probably continues for a considerable time, as the stage is common in preparations.

In the polymitosis just described the original karyosome—and the daughter-karyosomes, if any—plays no active part. It merely degenerates and breaks up, and its fragments are finally absorbed by the cytoplasm in which they are liberated. (Cf. Figs. 43, 49, 50, 51, 53, 54, 58.)

It is clear that this peculiar division is a kind of multiple mitosis. The original nucleus contains six differentiated chromosomes (*a–f*), and these divide equally into two for a number of times in succession. The daughter nuclei which finally result are thus each provided with a set of six similar chromosomes (*a–f*), and are therefore all equivalent. No reduction of the chromosome number takes place. The same number of chromosomes is present originally in males of all sizes, and the daughter nuclei—whether many or few—are identical in chromosome content in all forms.

The resting nuclei (Fig. 59) vary somewhat in size, being rather larger in large organisms than in small: but the difference is not great. Usually they are oval, the longer diameter lying parallel to the surface. Their sizes range from about 3–4 μ by 5–6 μ down to about 2–3 μ by 3–4 μ.

From this stage onwards the nuclei continue to divide by repeated binary mitosis—only the first division, just described, being a polymitosis. Nuclear divisions continue until the entire surface of the organism is uniformly studded with small nuclei. Small males remain more or less globular throughout, but large ones undergo a variable amount of superficial folding—the folds, in very large individuals, ultimately becoming deep clefts which cut the organism into several pieces. Apart from these differences parasites of all sizes develop in the same way.

These later nuclear divisions are all effected in a similar manner. The nuclei increase in size, after the first resting period, and become spherical. They then usually lie in slight superficial elevations of the cytoplasm (Fig. 60), which become more and more salient during the later stages. At the summit of each elevation an achromatic cone can be distinguished, projecting as a minute nipple above the surface (cf. the two nuclei on the right in Fig. 60). In the prophases of the ensuing mitosis the reticulum gives place to a spireme in the usual way, and this then breaks into filamentar chromosomes. As soon as they are formed, they pass in a bunch to the achromatic cone, and attach themselves to it. (The lower right-hand nucleus and the lowermost nucleus in Fig. 60 show stages in the formation of the spireme: the upper left-hand nucleus shows the bunch of chromosomes attached to the achromatic cone.) The achromatic cone now divides into two, and its halves draw apart. Simultaneously the chromosomes all split longitudinally. One daughter group follows each cone (uppermost nucleus in Fig. 60), and as the cones diverge a characteristic spindle-figure is formed (lower left-hand nucleus in Fig. 60). The nuclear membrane becomes invisible during the formation of the spindle, but the nuclear area is generally distinguishable from the surrounding cytoplasm. Finally the cones and daughter-groups pull apart completely, and give rise to resting nuclei in the same way as the groups resulting from the polyaster.

During all these stages the chromosomes are difficult to count, owing to the circumstance that they are filamentar throughout, and bunched together at the time of splitting and separation. It is only when they separate completely at the late anaphases (Fig. 61) that I have succeeded in counting them with certainty and recognizing all the individual members (*a–f*) of the group. During a great part of the division, however, the *a* and *b* chromosomes are recognizable by their greater length. It will be observed that the mitosis is peculiar, as there is no equatorial plate stage, and the splitting of the chromosomes ("metaphase") occurs before a spindle-figure is formed. These divisions are, in fact, like simplified versions of those which occur at the preceding polymitosis.

Later divisions occur in the same way: but as the nuclei become more closely packed together on the surface, they become smaller, and are unable to draw far apart at the anaphases. For these reasons the chromosomes become increasingly difficult to recognize and count. Fig. 65 shows a typical pair of anaphase groups during the later divisions: and although all the chromosomes are not clearly recognizable, *a* and *b* can be made out, and I have no doubt that all such groups really retain their six constituents throughout.

Except at the first division after the resting stage following the polyaster (Fig. 60), the nuclei stain deeply and readily, and appear to be rich in chromatin. During all the later divisions they have a peculiar appearance[1] when at rest (Fig. 62), suggesting that the chromosomes persist in a differentiated condition

[1] This appearance was noted by Siedlecki (1898), whose Fig. 10 (Pl. VII) is evidently intended to show it.

all through the "resting" period. In nuclei such as those shown in Fig. 62, however, I am unable to distinguish individual chromosomes with certainty.

As the nuclei become more crowded, in the last divisions of all, they form extremely short spindles—in the same way as before—but the whole division takes place inside the persistent nuclear membrane (Figs. 63, 64, 66). At the anaphases the chromosomes are relatively shorter and thicker (Fig. 66) and can sometimes be counted with some certainty: but in the earlier stages (Figs. 63, 64) I have been unable to enumerate them. The achromatic cones persist throughout these divisions and the intervening resting stages (Figs. 60, 62–66), and behave as division centres. They never appear to me to contain centrioles within them, but to be themselves, in their entirety, comparable with centrioles or centrosomes.

Fig. 66 shows the late anaphases of the last nuclear division before gamete formation. Some of the chromosomes are still clearly recognizable (*e.g.*, in the right-hand group *a* and *b*, and probably *c* and *d*), and from the general appearance of the nuclei at these stages I have no doubt that the typical complex of six chromosomes is, in reality, always present: but this is a matter of inference, and not of observation. During the later divisions, the smallest chromosome (*f*) can rarely be distinguished with certainty. I conclude, however, that it is always present throughout, because it is distinguishable in the earlier stages[1] (polyaster, and next divisions) and there is no reason to suppose that it drops out during subsequent divisions.

In spite of the difficulty and uncertainty in counting the chromosomes during the later divisions in the male, I think it must be assumed that the chromosome number remains the same throughout. It is certain, in any case, that the number is not halved during these divisions, and there is good evidence that it remains constant—even if the constancy cannot be proved in every nucleus. I have examined these stages with great care, and am convinced of the essential truth of these statements: but the later nuclear divisions are so difficult to study, and the nuclei themselves are so small and complex, that I should speak with far less confidence were I ignorant of the constitution of the earlier nuclei and of the subsequent development.

When the last nuclear divisions have been effected the male forms its microgametes—one nucleus entering into the formation of each. This process will be described in the next section: but before I describe the microgametogenesis of *A. eberthi*, I must refer briefly to certain observations of others on the phenomena just described.

I need not discuss the earlier observations—by Schneider, Mingazzini, Labbé, etc.—on the nuclear divisions in the male parasite, for they were all obviously based upon misunderstandings of various sorts. The first approximately correct account is that of Siedlecki (1898), whose observations are easily understood from what I have already said. He saw various stages of

[1] That it is present also at later stages in the microgamete nuclei is evident from the chromosome counts of zygote nuclei—to be described later.

the polyaster, but did not find the first spindle. Consequently, he drew the erroneous conclusion that the whole nucleus resolves itself into "filaments" which mass themselves subsequently in irregular groups at the surface of the organism and there form a number of nuclei. These "filaments" were, of course, the chromosomes (which he did not count). His Figs. 6 and 7 (Pl. VII) show abnormal individuals, and not stages in nuclear division: his Fig. 8 depicts the stage where the chromosome groups emerge from the polyaster (intermediate between my Figs. 57 and 58): but his Fig. 9 shows an organism at a much later stage, when the nuclei are dividing by bipolar mitosis after the period of rest (a stage which he seems to have overlooked). Siedlecki's observations are, in general, correct, but he failed to make out many details—which is hardly to be wondered at, considering that he was a pioneer.

Moroff (1908) has described and figured—under various specific names—several stages in the nuclear divisions of the males of *A. eberthi*; but his description is obviously based upon an incomplete series of specimens, some of which were certainly abnormal and degenerate. Except that he gives a partial picture (his Text-fig. J. 1, p. 108) of an early polyaster, he adds little that is both new and accurate[1] to the earlier account of Siedlecki. The same may be said of the work of Pixell-Goodrich (1914).

(5) *Formation of the Microgametes.*

After the final nuclear divisions have taken place in the male parasite, its whole surface appears closely and uniformly studded with numerous small nuclei (Pl. III, fig. 69), each of which is spherical and vesicular, with a diameter of about 2·5μ. As they enter the resting state, after the last division, these nuclei undergo a slight reduction in size. The telophase chromosomes break down to form a finely granular network, and simultaneously the surrounding nuclear membrane becomes more evident (Fig. 69 shows several stages).

Each nucleus forms the middle of a mammiform boss, sharply projecting from the surface of the organism—each boss being surmounted by a minute nipple which is formed by the persistent achromatic cone (Fig. 69). The mammiform processes soon begin to show a slight constriction at their bases, and at about this time—sometimes a little earlier or later—two flagella begin to grow out from each nipple (Fig. 70). These, when first formed, are very short and fine. As a rule they grow out simultaneously from the tip of the cone: and when only one is visible—as sometimes happens (cf. Fig. 70)—it is, I believe, probably owing to some imperfection in staining or difficulty of observation.

The nuclei now begin to grow smaller and more compact (Figs. 70, 71), and assume a somewhat reniform appearance which becomes gradually more

[1] Moroff's description here, and elsewhere, is to me so confusing—on account of his attribution of different stages to distinct species, and my difficulty in separating his observations from his speculations—that I find it very hard to ascertain exactly what he did observe in *A. eberthi*. The above is my considered opinion: and if it is unjust, the injustice is unintentional and due to my inability to understand his descriptions.

definite (cf. Figs. 70, 71). At the same time the basal constriction becomes more conspicuous, and by the growth of the cytoplasm in this region a short stalk is formed—the nucleus thus coming to occupy the thickened end of a short club-like outgrowth[1] (Figs. 71, 72).

These outgrowths are now recognizable as young microgametes, or spermatids (Fig. 72). By suitable staining it can be shown that they possess several structures not hitherto observable. At the base of the paired flagella a small deeply staining granule is now observable (Figs. 71, 72), from which the flagella evidently take their origin. This granule, or blepharoplast, is derived from the achromatic cone: but whether it actually is the same structure —more condensed—or only a part or derivative of it, I am not able to determine. At stages such as that shown in Fig. 70, no granule is demonstrable[2] with certainty at the base of the young flagella: but at the stage of Fig. 71 it is clearly visible. Moreover, this basal granule, at these later stages, always appears to be united to the nucleus by an extremely slender thread (Figs. 71–74), or rhizoplast. Comparison of these structures—blepharoplast and rhizoplast—with a centriole and central spindle at once suggests itself: but whether such a homology is justifiable I do not know, as I have been unable to demonstrate such structures at the last nuclear division, or to trace their continuity with the organs present in the spermatids.

Another structure which now first becomes visible is a small frill or membrane on each spermatid (Figs. 71, 72). This is the beginning of the undulating membrane, which is present in every mature microgamete. When first seen it appears as a slender lateral frill, with a darkly staining border (Fig. 72). This border appears to be a filament[3], which arises from the blepharoplast anteriorly, and posteriorly fades away into the neck or stalk of the spermatid. It closely resembles the marginal flagellum of a trypanosome. Precisely how it is formed I am not certain, as in its earliest stages it is excessively small and delicate. At the stage of Fig. 71 its first beginnings appear to be sometimes present; but it is not until the stage of Fig. 72 that it can be made out with complete certainty.

The final stages in the formation of the microgametes are shown in Figs. 73, 74, 75, and 76, which almost explain themselves. They consist in a great elongation of the whole spermatid, with a drawing out of its nucleus and undulating membrane, and a considerable increase in the length of the flagella. The nucleus becomes homogeneous, and undergoes peculiar changes in shape;

[1] This stage—but without any structural details—has been figured by Siedlecki (1898, Pl. VIII, figs. 12, 13): also earlier by Schneider (1883, Pl. VIII, fig. 17), who considered it as "un état cadavérique."

[2] The whole achromatic structure can, of course, be stained deeply by iron-haematoxylin methods—by overstaining: but on differentiation no central granule can be demonstrated unequivocally as a separate and independent organ.

[3] This structure was first noted, at this stage, by Siedlecki (1898), who wrongly described it, however, as an *axial* filament lying in the cytoplasmic stalk of the spermatid, and arising from the nucleus.

becoming first somewhat cap-like (Fig. 73), then flattened and leaf-like (Fig. 74), and finally drawn out into an irregular filament (Fig. 75). Throughout these stages the blepharoplast can be distinguished, and its connexions with the nucleus, flagella, and margin of the undulating membrane can be made out (Figs. 72 to 75). The spermatids remain attached to the residual body of the male during all these stages.

The final conversion of the spermatids (Fig. 75) into mature microgametes, or spermatozoa (Figs. 76, 77), is accompanied by increase in length and still further attenuation. The fully-formed microgamete (Figs. 76, 77) is very long and thin and sinuous, and tapers to an excessively fine point at the posterior end. It is thus extremely difficult to measure accurately: but its width in the middle is about 0·75μ, and its length from 35μ to 45μ. Its body is circular in transverse section (Figs. 76*a*, *b*)—being cylindrical, not ribbon-shaped. The two flagella are of approximately equal length and are some 15μ long. (I am unable to determine their length exactly, as their ends are seldom distinctly visible. They are excessively fine threads, which are usually entangled with one another in cysts, and very difficult to distinguish when the gametes are lying in the tissues.) The filamentar nucleus occupies about the anterior two-thirds of the body (Figs. 76, 77). In transverse sections it is seen to lie laterally, and to be slightly channelled on its inner aspect (Fig. 76*a*). The undulating membrane is visible running almost the whole length of the body, but posteriorly it fades away into the caudal filament, which appears to be formed by the drawn-out body as a whole. The minute blepharoplast lies subterminally at the anterior end, and is difficult to discern except in unusually well-stained individuals. The nuclear filament reaches the extreme anterior end of the body, and a few bright but feebly staining granules can usually be seen lying in or on it at variable points in its length (Figs. 76, 77)[1].

When first formed the microgametes lie attached by their very slender tails to the residual bodies within the cysts[2]; and the cysts thus appear to contain one or more balls of protoplasm covered with extremely fine hairs (Pl. II, figs. 67, 68). After a time the gametes break loose, and swim freely in the cysts. Later the cyst wall ruptures, and they escape into the surrounding connective tissue.

Microgamete cysts vary very greatly in size and in the number of gametes which they contain—both being dependent upon the size of the original male organism. Nuclear divisions continue until the whole surface of the male is

[1] Dead or degenerate microgametes, lying in the tissues, show a variety of structures quite different from those described above. They are usually devoid of flagella, more or less swollen, and often clubbed at the ends. Their nuclei are vacuolate, or segmented or broken into granules of variable size. I may note that the microgamete is probably even more complex in structure than I have described it, for I describe only what I have been able to observe regularly and with certainty, and I am fairly sure that I have not been able to elucidate every detail. The microgametes are excessively difficult objects to study.

[2] It will be evident from what has already been said that the cyst is a complex structure derived partly from the pellicle of the parasite and partly from the tissues of the host.

completely studded with small nuclei; and as the nuclei finally formed are of the same size in males of all sizes, the number of microgametes ultimately formed is proportional to the surface area. A small male thus produces a small cyst, with one residual body and a few scores of microgametes (Fig. 67); whilst a large male gives rise to a large cyst containing several residual bodies and hundreds or even thousands of microgametes (Fig. 68). It should be emphasized that it is in their numbers, and not in their size, that the microgametes in different cysts vary. All microgametes, when fully formed, are of approximately equal size, and equivalent in structure and nuclear constitution.

As a rule it is impossible to count the microgametes present in a cyst. Figs. 67 and 68 (Pl. II) give a fair idea of their appearance, and of the difference in size which such cysts may show. (These figures were drawn from whole cysts, in stained smear preparations. Fig. 67 is from a cyst measuring about 60μ in diameter: the longer diameter of that shown in Fig. 68 is approximately 200μ.)

Fully-developed microgametes move actively, with a serpentine motion, and with the flagellar end in advance. After the rupture of the cyst they migrate in all directions into the submucous connective tissue of the gut wall, where enormous numbers of them are sometimes seen lying among the cells so thickly as to give the tissue a peculiar fibrillate appearance. Such "microgamete tissue," as I call it, usually contains microgametes in all stages of degeneration. How long they are able to survive, and how far they can wander, I have not been able to determine: but as it is often possible, in good serial sections, to find near at hand the remains of the cyst from which a given group of microgametes has escaped, and as microgametes which are not near such a recently disrupted cyst are usually degenerate or dead, I conclude that most of them do not live long in the connective tissue, and that the migrations of the majority are not usually extensive.

Neither the microgametes themselves nor the details of their formation have been accurately described or figured hitherto. The microgametes were first seen—though not understood—by Eberth (1862)[1]; but Siedlecki (1898) was the first observer to interpret them correctly, though his account of their structure was imperfect. According to him a ripe microgamete consists almost entirely of chromatin, and possesses no flagella[2]. He described no undulating membrane, but to judge from his figures[3] he probably observed this structure. A more detailed account of the much larger microgametes of an *Aggregata* from *Octopus* was given by Moroff (1908), whose description and figures agree in several respects (flagella, nucleus) with my observations on *A. eberthi*. Pixell-Goodrich (1914) added some further details to Siedlecki's description and observed the flagella; but her figure[4] of the microgamete of *A. eberthi*

[1] He called the ripe microgametes surrounding the residual body a "gregarine thickly clothed with hairs."

[2] This statement is italicized in the original.

[3] Siedlecki (1898), Pl. VIII, fig. 16.

[4] Pixell-Goodrich (1914), Pl. XIII, fig. 11.

shows a gamete which is bluntly rounded at either extremity, and furnished with two[1] very conspicuous blepharoplasts. Its body is said to be "strap-shaped, with a more or less cork-screw nucleus running through the anterior half."[2] An undulating membrane was observed and figured at the hind end only. Little or nothing is said concerning the development of all these structures, and it will be evident that the description does not agree with my observations.

The views of Mingazzini, who first (1890) regarded the microgametes as the sporozoites of a different species, and later (1892, 1893) considered them to be "swarm-spores" (or merozoites) of *A. eberthi*; and the views of Schneider (1883) and Labbé (1896), who considered them to be degenerative or teratological products[3], require no discussion at the present day. Beyond doubt all these notions were profoundly erroneous, as Siédlecki was the first to show.

(6) *Fertilization.*

We have already traced the development of *A. eberthi* up to the moment when the macrogametes (Pl. I, fig. 34) and microgametes (Pl. III, figs. 76, 77) are formed. The process of fertilization consists, essentially, in the fusion of one microgamete with one macrogamete: but in *Aggregata* the events which immediately follow this fusion are so complex, and lead so insensibly into the phases of meiosis (reduction) and the first nuclear divisions of the sporont, that to separate these continuous events into definite stages is an arbitrary procedure. It is adopted here merely for convenience of exposition—the term "fertilization" being used to denote all those stages which occur from the time when the microgamete enters the macrogamete up to the time when the fusion of the two appears to be complete.

The mature macrogamete (Pl. I, fig. 34) is large and rounded; but its nucleus is oval or somewhat fusiform, and lies with one of its poles in contact with the surface. At this pole the chromatin granules derived from the chromosomes lie in an irregular heap, suspended by a few linin threads attached to the nuclear membrane: while fragments of the karyosome, in process of degeneration, usually lie scattered irregularly through the rest of the nucleus, though occasionally a single large unfragmented karyosome is still present at this stage.

Only a few microgametes succeed in encountering macrogametes and fertilizing them: all the rest perish. There is not much evidence that the microgametes are specially attracted by the macrogametes. It is true that they are often to be seen massed around ripe macrogametes; but they are also equally often seen similarly congregated round male parasites, unripe macro-

[1] Number not stated in the text. Moreover, the structures figured are in a gamete stained with Delafield's haematoxylin—a stain which certainly does not usually demonstrate such granules.

[2] *Ibid.*, p. 165.

[3] Labbé (1896), p. 615, asserted that "there can be no doubt" that the microgametes "are teratological formations." It is difficult to ascertain the grounds for this and many another confident but erroneous opinion expressed by this writer.

gametes, and spores or sporoblasts. In their migrations through the tissues the microgametes frequently encounter other parasites in all stages of development, and they may enter and even pass through the dead ones. I have more than once observed cysts containing degenerate sporoblasts mixed with microgametes[1]; and as dead cysts are usually invaded by phagocytes also, the appearances presented by such mixtures are often very puzzling. It is noteworthy that the microgametes do not, as a rule, enter the mucous membrane or the muscular coats of the intestinal wall.

Experiments which I made to determine whether the macrogametes attract the microgametes were inconclusive. Mixtures of ripe microgametes and other stages (female parasites, macrogametes, sporoblasts, spores, etc.) examined alive under the microscope, invariably gave me disappointing results. I made these experiments originally in order to determine directly the stage at which fertilization occurs, but I was never able to observe this process *in vivo*. As a rule, indeed, the microgametes remained almost motionless: and when they did show any activity, they appeared to have no "affinity" for any particular stage or kind of parasite. I have no doubt—after repeated failures—that in order to succeed in experiments of this sort it is necessary to have great luck. One must have the good fortune to examine just that area of the intestinal wall which happens to contain microgametes and macrogametes which have just reached maturity at the moment of the experiment. It is remarkable how seldom an area of this sort—showing all stages in fertilization—is met with in sections of the gut wall, though ripe gametes, of either sort, are common.

The microgamete enters the macrogamete at the point where the nucleus of the latter touches the surface; and it thus passes directly into the nucleus itself (Pl. III, fig. 78), and becomes imbedded in the heap of chromatin granules lying near the point of entry[2]. The penetration of the microgamete is probably very rapid, because these stages are extremely scarce in preparations, though those immediately before and after are not uncommon. Probably the microgamete enters with its anterior (flagellar) end in advance, because its nucleus lies at this end and can be traced—as a deeply staining filament—right into the chromatin granules of the macrogamete's nucleus (Figs. 78–80). It is probable also, I think, that the macrogamete forms a "cone of reception" for the microgamete: but I have seen this in only a single specimen, which may possibly be abnormal[3]. This specimen (Pl. III, fig. 79) is a very small

[1] I mention this particularly because such cysts may appear, at first sight, to confirm Moroff's statement that fertilization occurs at this stage.

[2] In Fig. 78 the macrogamete is seen somewhat obliquely from the pole of entry, and the chromatin granules and microgamete are actually lying much nearer the surface than they appear to be. The position of these structures is really similar in Figs. 78 and 80, but appears different because the specimens are viewed from different angles.

[3] I believe—on analogy with other organisms—that the phenomenon is normal, but that the formation of the funnel and the entry of the microgamete take place so quickly that specimens showing this stage are excessively rare.

macrogamete lying in the wall of the caecum. The whole nucleus is very delicately stained, and contained in one thick section. At the point of entry, the microgamete appears as a slender filament, lying partly within the chromatin mass of the macrogamete, and partly outside it in a funnel-like depression. This conical pit (triangular in side-view) appears to lead directly into the apex of the nucleus, and to represent the path followed by the microgamete: and although the membrane forming the wall of the macrogamete is well developed elsewhere, it is absent, or possibly extremely attenuated, in the region of the cone.

Although I have been unable to observe the process in the living organism, I conclude from my preparations that the entry of the microgamete takes place as follows. Microgametes migrating actively through the connective tissue occasionally encounter a ripe macrogamete. Those which come in contact with the general surface of such a gamete are unable to penetrate its coverings (consisting of its own membranous wall or pellicle internally, and an external investment derived from the cells of the host). But if a microgamete is fortunate enough, after working its way through the tissues, to reach that point on the macrogamete where the outer pole of its nucleus touches the surface[1], it is then admitted. The wall of the macrogamete becomes excessively thin at this point, or disappears altogether, and a funnel-like depression leading to the nucleus is formed. Into this the microgamete immediately passes, flagellar end first. It then goes straight through into the nucleus, and the funnel closes up behind it.

As soon as the microgamete has entered the nucleus of the macrogamete two striking changes occur: the macrogamete surrounds itself with a "fertilization membrane"—a definitely delimited (double-contoured) wall—and simultaneously undergoes a change in the staining reactions of its cytoplasm. The formation of the fertilization membrane appears to take place suddenly—as in the eggs of some Metazoa—and to prevent the entrance of other microgametes[2]. The membrane itself appears to be a derivative of the original pellicle of the macrogamete. This pellicle, which closely invests the macrogamete before fertilization (cf. Fig. 34), becomes more distinct and slightly

[1] According to Pixell-Goodrich (1914) there is here "a plug of dense, finely granular protoplasm, which gives off radiations, spreading in all directions through the cytoplasm." I regard the appearances so interpreted as artifacts. Certainly no such structure is present in my specimens at the moment of fertilization, though similar appearances may be seen in occasional parasites—when badly fixed—at this and other stages. The figures illustrating the phenomenon are unconvincing. One (Pl. XIII, fig. 1) does not show the whole nucleus, so that it is not possible to judge what stage of development the macrogamete (?) has reached: the other (Fig. 2) shows a macrogamete which is clearly—from its nuclear structure—not one which (as stated) the microgamete "has just entered." Normally, at the moment of fertilization the nucleus of the macrogamete is not separated from the surface—which it touches—by any measurable interval, and there is thus no room for a dense mass of protoplasm at this point.—An artifact of a similar sort had been previously described and figured—as a normal occurrence, at a different stage—by Moroff (1908, pp. 116–17, Text-fig. O). The phenomenon is said to occur in "*A. arcuata* (?)," which is really *A. eberthi*.

[2] The formation of the fertilization membrane was first seen, and correctly interpreted, by Siedlecki (1898), who called it "la membrane de l'œuf."

thicker (Fig. 78), and then separates from the underlying cytoplasm, leaving a space filled with fluid between itself and the protoplasm (Fig. 80). In stained specimens the membrane is often thrown into folds (as in Figs. 80, 84), but this is probably the result of fixation. As the membrane separates, the protoplasm becomes denser: and I interpret this as a sign that the intervening space is formed—in part, at any rate—by the contraction of the protoplasm. At a later stage the fertilization membrane becomes fused with the cellular investment previously present, and the compound capsule so formed constitutes the oocyst.

The increase in density shown by the protoplasm immediately after fertilization is accompanied by a complete change in staining properties. Before the entry of the microgamete, the cytoplasm of the macrogamete shows, when fixed, large alveoli, and stains with "acidic" or "plasma" stains but not with "chromatin" stains. After fertilization, however, its reactions are reversed. The cytoplasm becomes denser, with smaller alveoli, and shows considerable affinity for chromatin stains. It becomes, in fact, in its staining reactions, closely similar to the cytoplasm of male parasites. Although this change is visible with all methods of nuclear staining—which show the fertilized macrogametes more deeply stained than the unfertilized[1]—it is particularly striking in preparations treated by some of the double-staining methods. For example, after appropriate staining with safranin and light green, all female parasites and unfertilized macrogametes are a more or less vivid green, whilst males—at all stages—and all fertilized macrogametes are coloured red or reddish. These appearances probably indicate that the protoplasm, at the moment of fertilization, undergoes an alteration in its state of aggregation and simultaneously becomes more acidic.

As the stage shown in Fig. 80 is not very uncommon, it probably persists for some time. The microgamete is at first recognizable as a somewhat granular filament, imbedded in the mass of chromatin granules belonging to the macrogamete; but it then begins to undergo a peculiar change. The original filament becomes shorter and thicker, and dilates at one end (Fig. 81[2]). Finally it becomes transformed into a minute spherical and vesicular body, which sometimes shows a central karyosome (Fig. 82). These stages occur very rarely in my preparations; but they are, in some specimens, so clear and definite that I think they must be normal. If my interpretation of them is correct, they would be accurately described by saying that the dense filamentar nucleus of the microgamete transforms itself into a delicate vesicular nucleus soon after its entry into the chromatin mass of the macrogamete.

[1] Siedlecki (1898) evidently observed this phenomenon, though he says little about it: for his figures clearly show it (cf. his Pl. VIII, figs. 17, 18, and Pl. IX, figs. 19–22), and he notes (p. 828) that "at the first stages following fertilization...the protoplasm...stains more intensely."

[2] In this figure the chromatin of the macrogamete is not shown in its entirety—for the sake of clarity, only an optical section of the nucleus at the level of the "sperm head" being depicted. In reality, the latter is entirely surrounded by chromatin granules, which are disposed in an irregular network.

But the nucleus of the microgamete does not retain its individuality for long. It soon becomes indistinct (Fig. 83) and breaks up, so that it is impossible to recognize it as a separate structure from this stage onwards. It appears to disintegrate into chromatin granules which become inextricably confounded with those of the macrogamete.

As already noted, the heap of chromatin granules of the macrogamete is suspended by linin threads from the nuclear membrane. These now begin to get thicker and more evident—one or two which pass to the ends of the nucleus being often specially conspicuous (Figs. 83, 84). At the same time the nucleus itself becomes greatly elongated, and gradually assumes the form of a spindle whose length may equal about five-sixths of the diameter of the whole organism, though it is not always so long. The linin threads also become more numerous, and the chromatin granules pass along them. A peculiar nuclear figure results—the so-called "fertilization spindle," previously observed in this organism[1] and other coccidia by many workers. Figs. 84, 85, 87, 88, 89, illustrate the formation of this "spindle" more clearly than I can describe it in words. (Figs. 88 and 89 show the spindle, when fully formed, in a small and a large parasite respectively—at the same magnification.)

A few further notes must be made on these stages. Nuclei such as are shown in Figs. 84–89 actually present a much greater variation in the disposition of the filaments than may be here apparent. After carefully studying a large number of similar specimens, I have come to the conclusion that there is no regularity in the number of the filaments or in their arrangement[2]. As a rule they mostly lie in the long axis of the nucleus and attached to its ends, but their points of attachment vary: and when the "spindle" is fully formed, most of the filaments lie against the nuclear membrane (Fig. 89). When the threads are forming, they appear loose and coiled, and in places studded with chromatin granules (Figs. 84, 85, 87): but when fully formed they appear as unbeaded and much straighter threads—some excessively fine, but others relatively thick (Figs. 88, 89). In fully-formed spindles (as shown in Figs. 88 and 89) the threads appear to be in a state of tension. The whole conformation of the nucleus suggests that its two poles are being forcibly pulled apart, with a resultant stretching of the contained filaments. The threads appear relatively thicker, and stain more deeply, in small organisms than in large (cf. Figs. 88 and 89): and this suggests that the amount of chromatin is approximately the same in nuclei of all sizes.

It is necessary to point out, perhaps, that the finer details of structure are extremely difficult to make out, and that the "fertilization spindle" cannot usually be accurately studied in whole (unsectioned) specimens: whilst

[1] First described in *A. eberthi* by Siedlecki (1898).

[2] In view of Reichenow's statements (1920, 1921) regarding *Karyolysus* I must point out here (1) that the fertilization spindle is not formed of individualized chromosomes in *A. eberthi*, and (2) that it is certain that no parallel pairing of the chromosomes occurs before, during, or immediately after this stage. Figs. 84–90 will, I trust, convince the reader of the truth of this statement.

individual sections of large nuclei at this stage often give an entirely wrong idea of the structure as a whole. It is also very difficult—owing to their delicacy—to fix and stain these spindle-figures satisfactorily, and consequently artifacts, at all stages, are commonly seen in preparations.

From the moment when the microgamete enters the nucleus of the macrogamete until the time when the "fertilization spindle" is fully formed, the karyosome of the macrogamete degenerates. One large karyosome may be present throughout (cf. Fig. 80), though at other times two (Fig. 89) or a variable but often large number of small ones are seen (Figs. 83, 87, 88): but all of them sooner or later undergo fragmentation and degeneration. They play no part whatsoever in fertilization and in the formation of the "spindle[1]," which is derived entirely from the chromatin granules and linin threads of the macrogamete's nucleus augmented by the addition of the nucleus of the microgamete. The main karyosome, when it persists entire during these stages, is always seen—in suitably stained thin sections—to be empty; whilst its cortex usually stains less intensely and shows numerous vacuoles—usually lying near its inner and outer surfaces (Fig. 86). Often a few bright granular inclusions can be seen in the cortex, and occasionally a few brownish grains of pigment. Sometimes also a few feebly staining residual granules (cf. Pl. I, fig. 27) can be still seen in the interior of the karyosome. All these appearances are obviously signs of degeneration, which may be said—speaking generally—to consist in vacuolation, fragmentation, and loss of chromaticity.

Fertilization and the formation of the "fertilization spindle" in *A. eberthi* have been previously described by Siedlecki (1898), who has illustrated the process with three figures (his Pl. IX, figs. 19, 20, 21). His account, though in the main correct, requires considerable amplification and some correction—as the reader, if he accept the foregoing description, can see for himself. I will only note here that Siedlecki's Figs. 19, 20, and 21 (Pl. IX) are evidently drawn from sections[2], and do not show the whole nuclear structure: while the mass of threads interpreted as the nuclear chromatin of the microgamete (Fig. 19) is in reality the mixed chromatin of both gametes. From the presence of chromatic threads at the other end of the nucleus[3] it is clear that the organism shown is incomplete—the figure illustrating a section which does not contain the whole nucleus. I have no doubt that the figure is a partial picture of the nuclear condition illustrated in my Figs. 85 and 87 (Pl. III).

[1] Pixell-Goodrich (1914) says (p. 166) that chromatin streams out of the karyosome on to the "spindle" after the entry of the microgamete; but this is undoubtedly an error. The figure supposed to illustrate the process (Pl. XIII, fig. 2) shows, in reality, a "spindle" in which the karyosomes are disintegrating in a somewhat unusual manner.

[2] Pixell-Goodrich (1914) says that Siedlecki "worked chiefly with films," but he does not say so himself and he states specifically that he also used sections (pp. 801, 802, 803): and that he did so is clear from his descriptions and figures.

[3] Siedlecki (1898) wrongly supposed that the chromatin of the macrogamete is situated, at the moment of fertilization, at the opposite pole of the nucleus to that at which the microgamete enters. He reached this conclusion, I believe, because he never succeeded in finding the earlier stages in the process. All his figures show stages which occur after the microgamete has disappeared.

Moroff (1908) denied that any fertilization occurs at this point in the life-cycle, and such stages of the process as he saw he misinterpreted as prophases of the first nuclear division of the female parasite. Pixell-Goodrich (1914), on the other hand, adopted Siedlecki's interpretation, but added no new facts to those which he had previously discovered. She gives two figures (Pl. XIII, figs. 2 and 3) illustrating the process of fertilization. The first is labelled "Fertilization. The microgamete (*m.*) has just entered": but it shows a much later stage—the transition from the "spindle" to the "cobweb" figure (described in the next section), long after the microgamete nucleus has completely disappeared. The other figure (her Fig. 3), said to show "a specimen soon after fertilization," shows an early stage in the formation of the "fertilization spindle" (*i.e.*, an earlier stage than the preceding figure). It is evident that neither of these observers possessed adequate material on which to base a description of fertilization in *A. eberthi*.

(7) *The Meiotic Phases.*

In the preceding section I have arbitrarily restricted the term "fertilization" to all the events which occur from the entry of the microgamete until the complete formation of the "fertilization spindle." No break in the sequence of events occurs at this stage, of course; but the "spindle"—to judge from its comparative frequency in preparations—probably persists for a considerable time. The stages which immediately follow may be regarded as the prophases of the first nuclear division of the fertilized macrogamete: and as this division is a reduction division[1], at which the chromosome number is halved, I shall include all the stages from the "spindle" to that at which the halving is definitely effected, in "the meiotic phases." This subdivision is merely for convenience, for it is difficult to determine the exact point at which "reduction" begins or ends.

The stage immediately following the "fertilization spindle" is one which I call the "cobweb stage," because it is characterized by the formation of an excessively fine nuclear network somewhat resembling a spider's web (Pl. IV, fig. 90). The nucleus becomes shorter, changing from a spindle to an oval figure; but its central end often persists for some time as a pointed diverticulum (as in Fig. 90). At the same time the stainable threads of the "fertilization spindle" become detached from the nuclear membrane, and take up a central position; but they remain, nevertheless, attached by a variable number of very fine linin threads—whose attachments are also variable—to the nuclear membrane. A few threads are also sometimes seen (as in Fig. 90) to be attached to the main karyosome—when still present. Fig. 90 shows the entire spider's web figure in a single thick section of a small specimen. It was

[1] As I have already stated elsewhere (Dobell and Jameson, 1915). I did not mention this in my preliminary note (Dobell, 1914) because I was still, at that time (December, 1913), in doubt about the details. After I had discovered the reduction phenomena in *A. eberthi* it took me the best part of a year to verify my original observations, and to make a sufficient number of chromosome counts, at all stages, to establish the facts with certainty.

drawn as accurately as possible, but the central part of the web is so complicated, and composed of such fine threads, that I am unable to guarantee absolute accuracy in every detail. In single sections through nuclei of larger parasites at this stage the appearances are often misleading, and a satisfactory reconstruction of the web from serial sections is almost impossible. At this stage the chromatin is in the form of minute beads scattered all over the network—like dewdrops on a cobweb. I have seen many stages intermediate[1] between the "spindle" and the "cobweb"—for they are fairly common—but I cannot illustrate them owing to lack of space.

At the "cobweb stage" another nuclear structure makes its first appearance. This is a minute "achromatic" body which is the rudiment of the achromatic spindle formed for the mitosis which is now about to occur. When first recognizable it is an extremely small spherical body, lying inside the nuclear membrane at the somewhat drawn-out peripheral pole of the nucleus. This body, in the earliest stage at which I have been able to recognize it, is shown at the apex (upper end in figure) of the nucleus in Fig. 90. At this stage it appears structureless, and stains feebly with nuclear stains. As it lies at the point at which the microgamete entered the nucleus, it is possibly a structure which is brought in by the microgamete: but as I have been unable to detect it at earlier stages—in spite of very careful search—I am unable to say whether this is so or not.

The cobweb figure now loses its connexions with the central end of the nucleus and begins to concentrate itself round the achromatic body, which rapidly increases in size. Figures such as that shown in Fig. 91 are then seen. The nuclear network becomes coarser and closer at this stage. (It is not drawn in its entirety in Fig. 91, which shows an optical section of the nucleus at the level of the achromatic body.) A little later the achromatic body is seen to be still larger, and to be assuming an oval or ellipsoidal shape[2]. It is now a very definite body, of considerable size; but it still appears homogeneous (Fig. 93). The chromatin network at this stage shows definite signs of differentiation into coarser filaments, usually much twisted and tangled. (Fig. 93 is again an optical section in the plane of the achromatic body. It does not show the whole of the chromatic network—part of which lies above and below the achromatic body.)

A little later the whole nucleus acquires a more spherical form and becomes closely applied to the surface of the organism. The achromatic body is thus situated now at the point of contact of the nucleus with the periphery of the

[1] It is important to distinguish the "cobweb" figure from the net-like figures which precede the "fertilization spindle." In reality they are quite different, but in sections which show only a partial picture of these stages they may easily be confused. All these stages, also, must be carefully distinguished from the "pseudospindle" figures (Fig. 32) preceding fertilization. In other coccidia similar stages occur, but they are invariably confounded with one another in the published accounts.

[2] A partial view of the nucleus at about this stage, and showing the achromatic body in its characteristic form and position, is shown in Moroff's Pl. IX, fig. 77 (1908).

organism: and although its outer aspect appears to lie actually on the surface, its inner face is still inside the nucleus. It has the characteristic appearance, in side view, shown in Fig. 92: that is to say, it appears as a plano-convex lens, with the convexity in the nucleus, the plane surface flush with the surface of the organism. At this stage, however, it sometimes appears more like a concavo-convex lens, as there is a slight depression on the surface of the organism at the point where it is situated (cf. Fig. 92); but this depression is not always seen, and may possibly be an artifact. The chromatin network now assumes a very definite form. It lies like a loose and rather tangled skein of thread immediately against or under the achromatic body, but apparently suspended freely in the nucleus. Whether it consists of a single thread or of several, I have not been able to determine with certainty. It appears usually as shown in Fig. 92, and apparently consists partly of a close net, partly of continuous open loops of thread. I believe this figure is formed, in reality, by a single continuous filament—contracted into a ball, but with a few loose loops. In sections which contain the whole figure—uncut—I have never been able to make out any free ends.

From this contracted figure the skein now opens out into a number of loops. These (Fig. 94) extend through the whole nucleus, and their ends become adherent to the nuclear membrane. The loops are formed by very slender beaded threads, and their exact arrangement is extremely difficult to make out and draw—especially towards the centre, where they are more or less entangled. Fig. 94 was drawn very carefully with the *camera lucida*. It shows the entire contents of a nucleus in a single thick section: but the loops actually lie at different levels, and their ends—as already noted—are really in contact with the nuclear membrane. Their disposition can only be properly appreciated by studying the actual specimen, with very careful focussing. After studying a number of similar stages, and attempting to draw them, I have come to the conclusion that 12 loops are always formed, though they are usually very difficult to count (cf. Fig. 94). They vary in length, but are apparently all continuous with one another. In any case, there are certainly always more than six loops, and as a rule not more than 12, in sections containing whole nuclei. Parasites of all sizes appear to contain the same number of loops; but in large nuclei, distributed through many sections, a satisfactory count is impossible.

This count (12) is confirmed when we come to the next stage (Fig. 95): for now the loops break apart as separate filamentar chromosomes, which can sometimes be enumerated with certainty. In all cases—such as that depicted in Fig. 95—in which the chromosomes are countable, there are 12, composed of six pairs. The complex *a–f*, present in the prophases of the first division in the male (Pl. II, fig. 42) and in the nucleus of the macrogamete before fertilization (Pl. I, fig. 31), is here again clearly recognizable; but instead of there being a single set of chromosomes (*a*, *b*, *c*, *d*, *e*, *f*), there are now two of each category. This is in exact agreement with

expectation[1]: for the nucleus now under consideration is a zygote nucleus ($2x$), into whose composition a microgamete nucleus (x) and a macrogamete (x) have entered—each of these gamete nuclei being known, from its previous history, to contain the complex *a–f*, consisting of six individualized and unpaired chromosomes.

It is usually very difficult to count the chromosomes accurately at these stages. They lie at different levels in the nucleus, and some of them are usually superposed or entangled. I have found it possible to make certain counts in moderate-sized individuals only, in thick sections containing whole nuclei. Very small parasites generally have their chromosomes too tightly packed or too entangled: whilst in large specimens they are so widely separated that the nuclei have to be reconstructed from serial sections. Even in a nucleus such as that in Fig. 95 the chromosomes are not easily appreciated—as regards either number or form—at a first glance[2]. As a result of making similar drawings of a number of other nuclei at this stage, I have come to the conclusion that 12 chromosomes (six pairs) are invariably present, though I am not always able to count them with certainty. Whenever I have been able to make a certain count, 12 were present; and when the counts were uncertain, the number was always approximately 12, and always greater than six. Variations in the counts have always been due, in my opinion, to technical difficulties involved in counting, and not to variability in the actual number of chromosomes[3].

It must be noted regarding the chromosomes at this stage that they are all filamentar: that they show very little beading, being as a rule almost smooth threads: and that they do not vary greatly in size (length or thickness) in nuclei of different sizes. (They appear, therefore, relatively larger in small nuclei.) The size-relations of the 12 chromosomes are constant. There are two very long ones (*a*), and two very short (*f*), while the rest form pairs of intermediate sizes. These relations are not always easy to make out, and may not be clearly evident even when the chromosomes are separately traced out and measured: because the filaments are really disposed in the nucleus in three dimensions of space, whereas in a flat drawing one dimension is eliminated. The true length of any chromosome is therefore seldom shown with absolute accuracy in a drawing.

Whilst the 12 chromosomes have become differentiated, the achromatic body has increased in size. The chromosomes now leave the nuclear membrane,

[1] Expectations did not influence my counts, however: for, in order of discovery, I actually determined the number of chromosomes at these stages before I had succeeded in determining the numbers present in male, female, and asexual individuals.

[2] Fig. 1, *F* (p. 85) in my preliminary note (Dobell and Jameson, 1915) shows the same nucleus but with the chromosomes labelled. It will therefore serve as a key to Fig. 95 if the reader finds difficulty in identifying the individual chromosomes in this drawing.

[3] It is hardly necessary to add that it is a mere waste of time to attempt to count the chromosomes in nuclei which are not really well fixed and stained: but I may also remark that it is by no means easy to obtain perfect preparations of these stages.

and clump themselves in an irregular mass in the centre of the nuclear space. They then travel, massed together, towards the achromatic body (Fig. 96). At this stage they are usually so entangled that I have found it impossible to count them with certainty. Fig. 96 shows their appearance in a typical specimen, but the chromosomes in the middle of the mass cannot be resolved with certainty. This figure also shows—near the opposite pole of the nucleus—a few linin threads which have persisted (as often happens) from the preceding "spindle" and "cobweb" figures.

As the chromosomes approach the achromatic body, it grows larger, and becomes barrel-shaped; and finally becomes a perfect achromatic spindle, on to whose equator the chromosomes pass. (See Figs. 96, 97, and 100.) The achromatic body, in its earlier stages, always appears to have a perfectly homogeneous structure, though later—as in Fig. 97[1]—it shows definite spindle-fibres in the middle. When the spindle is fully formed (Fig. 100) it is sharply pointed at both ends[2], and projects as a solid body above the surface of the organism. Neither asters nor centrosomes are usually visible at this stage, though astral radiations may appear soon afterwards.

From the manner of its formation, and its behaviour towards stains, I believe that the achromatic spindle is formed as a solid body. The spindle-fibres are formed first in the equatorial region, and gradually extend towards the poles: and at these poles no centrosomes or centrioles are demonstrable. It is true that with certain methods of staining (iron-haematoxylin) the ends of the spindle may be more deeply stained: they may even be made to appear as irregular or round black lumps. But with proper differentiation, and with other methods of staining, no polar structures can be distinguished. When the spindle is fully formed, indeed, the fibres can be traced almost to the very poles, and it is impossible to find any structure there corresponding to a centrosome. Indeed, there appears to be no room for such a structure unless it be ultramicroscopic. I believe, therefore, that the "centrosomes" which are at times visible during these stages are artifacts. Moreover, when the astral radiations begin to appear—at the stage just described, or a little later—the spindle has a curious structure, which is shown in Fig. 99. This figure does not show all the chromosomes, and it shows the spindle in optical section. It will be noticed that its ends are formed of two large stellate masses connected by fibres, of which the central one is the thickest. The whole of this structure stains feebly, and although the central fibre suggests a centrodesmose, no centrioles are demonstrable in the asters. The whole of such a structure

[1] In this specimen the achromatic body—now showing differentiation of spindle-fibres—lies, in reality, in the usual superficial position: but it appears to lie more deeply in the nucleus, owing to the way in which the nucleus has been sectioned. The chromosomes, moreover, are not all present in this section.

[2] The spindle at this stage appears to have been first seen by Labbé (1896): but his account (p. 605) and figures (Pl. XV, figs. 1–10) are highly inaccurate. He describes the chromosomes as "usually very fine and very numerous," but his pictures—being unlike anything I have ever seen in *Aggregata*—are to me quite inexplicable.

appears to be formed of a single homogeneous "achromatic" substance, which stains a uniform gray with iron-haematein, uniform green with safranin-light green, and comparably with other methods.

From the study of spindles at all stages, and after fixation and staining in a variety of ways, I conclude that no centrosome, centriole, or comparable structure exists: or alternatively, if it does exist, then the whole aster must be regarded as a centrosome; and as this is only the differentiated end of the spindle, the whole achromatic structure, from the time of its first appearance as a minute body at the "cobweb" stage, must be regarded as a centrosome or its derivatives[1].

As the chromosomes pass on to the equatorial plate (Figs. 98, 100) they are still filamentar; but they are usually so crowded that they are very difficult or impossible to count. (In the specimen illustrated in Fig. 100 the 12 chromosomes can just be distinguished.) As they gain the equatorial plate they become shorter and thicker, and ultimately lose their filamentar form entirely and condense into more or less rounded masses. During this transformation they present a variety of curious shapes (Fig. 101)—many of them becoming clubbed and otherwise swollen or deformed. At this stage they are closely packed, and usually impossible to count. But very soon, as they become more regular in shape (Fig. 102) they can be more clearly distinguished once more: and it is then seen that they are associating in homologous pairs. The two large chromosomes (a, a) pair, and the two small ones (f, f), and likewise those of intermediate size. At the time of pairing they may be globular, somewhat rod-shaped, or slightly irregular, but the members of any pair are usually alike in form and size.

It is very difficult to study the chromosomes at these stages, and still more difficult to depict them; for they lie close together on the equatorial plate, and the larger ones therefore mask the smaller (Fig. 102). Moreover, the partners forming a pair frequently lie directed towards opposite poles of the spindle, so that in sections across the spindle (equatorial plates) one member of a pair often lies over its partner, and the paired condition is thus not evident. In side views of equatorial plates, on the other hand, the chromosomes often appear as an almost solid mass (Fig. 104). I have tried to illustrate a typical spindle at this stage by an artifice. A whole spindle is very carefully drawn in Fig. 102, as it appears in side view. The chromosomes, since they lie over one another, cannot all be thus shown, though they can all be made out with certainty, by careful focussing, in the actual specimen. I have therefore drawn each individual chromosome element separately[2]—as accurately as possible,

[1] The conditions here are closely similar to those already described during the first nuclear division in the male, and the above argument is therefore equally applicable to both.

[2] This was comparatively easy, as the specimen is stained with safranin—which is always a very transparent stain—and the chromosomes are, in reality, all separated from one another—not touching, as they appear in the composite picture (Fig. 102). I drew this same group of bivalent chromosomes in Fig. 1, *G* in my preliminary note (Dobell and Jameson, 1915), but they are there shown grouped together, and not in a row. The true arrangement I find it impossible to represent in a single drawing.

with *camera lucida*—in Fig. 103, which thus gives a faithful series of portraits of all the chromosomes at this stage. The six bivalent chromosomes, formed by the homologous pairing of the 12 original univalents, are here very clearly visible—with the possible exception of the *d* pair, whose double character is perhaps not self-evident: but it is almost impossible to find a nucleus in which all the pairs are lying in a favourable position for drawing.

Judging from their scarcity in preparations these stages do not last long. The bivalent chromosomes soon swell up into irregular vesicles, resembling small nuclei (Fig. 105). Disjunction of the *f* (smallest) pair may occur at this stage (as shown in Fig. 105), though it usually takes place later. Even at the earlier stages (Fig. 102) the homologous chromosomes are in close apposition, but in the vesicular state their bivalent character may disappear completely, though they generally appear more or less irregularly lobed. To elucidate this figure (105), I have again drawn each chromosome separately (Fig. 106).

The appearance of the chromosomes at this stage is so remarkable that for a long time I imagined that it was referable to some peculiar artifact or abnormality. The chromosomes appear more like nuclei; for they stain less intensely, and instead of being homogeneous they show a very definite reticular structure. If I could misuse terms in this way, I should say that each pair of chromosomes forms a small vesicular nucleus, of irregular shape, on the equatorial plate. I have seen this stage so many times now, and in specimens which appear otherwise so normal, that I am forced to regard it as normal also.

The chromosomes do not persist for long in the vesicular state. They soon condense into more solid deeply-staining masses again, and then each divides by constriction into two equal parts. The result of this division is the complete disjunction of the six bivalent chromosomes into two homologous groups of six univalents each (Fig. 107). Each group contains the typical set of six differentiated chromosomes (*a–f*), now more or less globular but definitely distinguishable by their sizes. As soon as the separation occurs the chromosome groups begin to travel towards opposite poles of the spindle.

The chromosome number is now "reduced" or halved from 12 to 6 once more, and at this stage the meiotic phases may be said to end—for descriptive purposes. As a matter of fact a complicated series of changes begins at this point—no "resting" nuclei being formed from the products of the first spindle: but I shall describe these, in the next section, as stages in the post-meiotic nuclear divisions of the sporont.

Nothing has yet been said about the behaviour of the nuclear membrane. It usually persists until the spindle is fully formed, and then vanishes; but it does not always disappear all at once, and the moment at which it disappears varies. As a rule it has gone completely at the stage shown in Fig. 100, where the chromosomes are passing on to the spindle. But at this stage, and for long afterwards, the area originally occupied by the nucleus can be distinguished from the surrounding cytoplasm by its staining properties and more homo-

geneous structure. The membrane itself disappears but the protoplasmic substance of the nucleus persists for a long time, becoming gradually merged into the cytoplasm.

Throughout the meiotic phases the karyosomes continue to degenerate and disintegrate. The main karyosome often remains recognizable and intact up to the time when the spindle is fully formed, and with the disappearance of the nuclear membrane it may come to lie freely in the cytoplasm. Smaller karyosomes, showing various conditions of degeneration and fragmentation, are commonly seen scattered irregularly through the nuclear area at all stages. They appear as homogeneous globules or droplets of variable size, and they are often a source of trouble; for they may get mixed up with the chromosomes and often lie near (or sometimes actually on[1]) the spindle, where they can be mistaken for chromosomes. They can usually be detected, however, by their form, their distribution, and their staining reactions. I have found no evidence, in good preparations, that the karyosomes are ever thrown out. They always break up gradually into smaller and still smaller fragments in the cytoplasm, and are finally absorbed. And I must here emphasize the fact that the karyosome (or karyosomes) of the macrogamete plays no part whatever in the processes of fertilization and reduction. Throughout the whole of these stages it degenerates, and its fragments are passively and irregularly distributed through the protoplasm.

Reduction has not previously been described in *Aggregata*[2], and the events which occur at this phase have either been overlooked by previous workers or regarded—as by Moroff (1908)—as stages in an ordinary mitotic division. The "chromatin reduction" described and figured by Labbé[3] is not a meiotic phenomenon at all, but a misinterpretation of appearances seen in certain degenerate and badly fixed individuals. It is unfortunately true, as Siedlecki has remarked[4], that Labbé's figures of *A. eberthi* are mostly "très inexactes." Siedlecki himself evidently never saw the meiotic phases. He says[5] it is very difficult to answer the question whether any reduction phenomena occur in *Aggregata*, and suggests[6] that the dissolution of nuclear chromatin which precedes fertilization, and its subsequent liberation into the cytoplasm (in reality, stages in the disintegration of the karyosome), may be regarded as a kind of "chromatic reduction." With reduction properly so called these phenomena, of course, have nothing whatever to do.

Some further discussion of the nuclear events occurring at fertilization and meiosis will be found on p. 116. I believe that the account which I have just given is essentially correct, but many of the nuclear figures are extremely

[1] I am not absolutely certain about this. When I have found bodies, which I believe to be karyosome fragments, on the spindle, but which from their size and shape I have been unable to distinguish with certainty from chromosomes, I have rejected such specimens and regarded the chromosome counts as doubtful.

[2] With the exception, of course, of my own preliminary note (Dobell and Jameson, 1915).

[3] Labbé (1896), p. 599, and Pl. XV, figs. 10, 14–17.

[4] Siedlecki (1898), p. 811 note. [5] *Ibid.*, p. 827. [6] *Ibid.*, p. 828.

complicated and difficult to study and interpret. All the stages described certainly occur, and they can be read in a single unbroken series. When allowance is made for faulty technique and abnormalities of various sorts, all my observations are consistent. Parasites of all sizes show the same stages and give the same chromosome counts whenever accurate counting is possible. The order in which the stages follow one another can be determined with considerable certainty: for only one sequence—that which I have described—forms a consistent series embracing all the forms here figured and their numerous intermediates. The moment of fertilization can be accurately determined by the entry of the microgamete, the change in the staining reactions of the macrogamete, and the formation of the fertilization membrane. The "fertilization spindle" is another fixed point, and the changes leading to its formation are comparatively easy to follow. Thereafter the gradual development and differentiation of the achromatic spindle supplies a valuable indication of the sequence of the other nuclear events, while the gradual disappearance of the nuclear membrane and disintegration of the karyosomes afford collateral evidence of the true order of succession of the various stages.

(8) *Development of the Sporont.*

Under this heading I shall include all stages of development from the early anaphases of the first nuclear division in the sporont up to the stage where the sporoblasts are formed.

We left the chromosomes—in the last section—at the moment when they separate at metaphase, on the first spindle, into two homologous groups of six differentiated (*a–f*) chromosomes each (Pl. IV, fig. 107). From now onwards the nuclear divisions are closely similar to those already described in the development of the male.

At the metaphase of the first division the chromosomes are always more or less globular and compact (Fig. 107)—just as they are at the first division in the male (cf. Pl. II, figs. 48, 49)—and they stain sharply and deeply. They do not always all divide simultaneously, the small (*f*) chromosomes being the most irregular in their behaviour. Sometimes this pair undergoes disjunction long before the others (Fig. 105), sometimes it divides late (Fig. 107): but most commonly the *f* chromosomes separate early, and precede the others as the anaphase groups pass towards the poles of the spindle (Fig. 108). As the early anaphases set in, all the chromosomes gradually elongate into a filamentar form once more, but at first they show great irregularity of shape. Figs. 108–112 show some of the appearances seen at these stages. (All these figures were made from parasites in which the chromosomes could be counted with certainty.) Fig. 108 is an early anaphase, with two groups of six chromosomes each (*a–f*)—all, except *f*, being more or less filamentar. Fig. 109 shows the chromosomes only, drawn separately, from an anaphase group similar to those shown in Fig. 108. Fig. 110 is a polar view of an anaphase group from another specimen. Fig. 112 is a slightly oblique view of a chromosome group—and one

end of the spindle—in a very large parasite. The *a* and *b* chromosomes are elongated, thickened, and twisted: the others (*c–f*) are still globular. Fig. 111 is a polar view of a later anaphase group, in which all the chromosomes are now filamentar. (In Figs. 110 and 111 they are longer than they appear, as they are in reality lying perpendicularly to the plane of the drawing.)

During all these stages the achromatic spindle is clearly differentiated. Its poles usually rise somewhat above the surface of the organism (Figs. 104, 108, 113), and send astral radiations deep into the adjacent protoplasm[1]. As the asters separate, the chromosomes become very irregular, and appear to be drawn out along the spindle-fibres (Figs. 113, 114, and 115)—just as in the first division in the male. In consequence, they soon become uncountable (*e.g.* Fig. 114).

Division of the asters now occurs at the poles of the spindle; and by repetition of this process, accompanied by splitting of the chromosome filaments, a polyaster figure—similar to that of the male—is formed. But this figure, though it shows great variation in form—according to the size of the organism—rarely becomes as complex and tangled as in the male. In very small organisms, the first division may end in the formation of only four nuclei (Figs. 116, 117): larger ones may, according to size, form six (Figs. 118, 119) or any other number up to about a dozen: very large specimens often form more than 20. The appearances of these stages are depicted in very small specimens (Pl. IV, figs. 117–119), and a large one (Pl. V, fig. 121) only. Intermediate-sized organisms show intermediate appearances, which will be readily understood, I think, from the extremes figured. The long ends of the drawn-out chromosomes remain attached (Fig. 117) in small specimens until a late stage—as in the males. In large individuals (Fig. 121), however, the ends become free, and the divisions proceed independently at the surface of the organism. A central chromosome tangle, such as is usually seen in the male polyaster (Pl. II, fig. 56), is seldom visible in the early divisions of the sporont.

The final stages of this "polymitosis" are closely similar to those in the male. After a certain number of divisions has occurred, each aster with its attached group of chromosomes emerges as an independent unit, and enters into the formation of a resting nucleus at the periphery (Figs. 118, 119, 120, 131). The resting nuclei so formed stain feebly, but otherwise show the characteristic reticular appearance. It is noteworthy that the size of these resting nuclei is about the same in parasites of all sizes (cf. Figs. 130 and 131). They are usually somewhat flattened or irregular vesicles, measuring about 10μ in diameter, and as a rule they contain a variable number of small karyosomes.

Just as in the case of the male polyaster, the chromosome groups, when they ultimately emerge to form resting nuclei, are clear enough for detailed study of their constitution. I have analysed and drawn a number of these

[1] The appearances at this stage have already been seen and figured by Moroff (1908): see, for example, his Pl. IX, figs. 78 and 79.

groups, and in every case where a certain count was possible they were found to contain the typical complex of six differentiated chromosomes. These can be clearly seen and counted in at least two of the nuclei in Fig. 119. Two other groups, at a higher magnification, are shown in Figs. 122 and 125. The chromosomes at these stages are filamentar, and usually have their ends looped or recurved (Fig. 122), so that they are by no means always easy to count. But with careful study, and with the use of the *camera lucida*, the counts can sometimes be made with considerable accuracy and certainty.

In large parasites the chromosomes can sometimes be resolved and counted at an even earlier stage, and their splitting can be demonstrated. Figs. 123 and 124 are drawn from a large organism—at the stage shown in Fig. 121—as accurately as possible in every detail. Fig. 124 is exact, but in Fig. 123 there is some uncertainty regarding the resolution of the filaments at the astral end. I am unable to count them here with certainty. The *a* and *b* (long) chromosomes are, as usual, more obvious than the others, and both are clearly split. I cannot see the *f* chromosomes in this group; but in Fig. 124 they are very clearly distinguishable, as they lie slightly in advance of the others.

Figs. 125 to 129 illustrate several stages in the formation of the resting nuclei from the separated polyaster groups. The telophases of these divisions present no unusual features. The chromosomes shorten and thicken, and then break up: a nuclear membrane appears, and the resting reticulum is formed in the usual way. During the telophases the aster loses its radiations and appears as a rather large homogeneous and solid body (Figs. 125, 127), which persists outside the nuclear membrane, and just below the surface of the organism, for a considerable time (Fig. 128). It gradually becomes smaller and smaller, until it disappears altogether: at least, I have usually been unable to find it in completely reconstructed resting nuclei (Figs. 129–131).

During all the foregoing stages the achromatic polar asters and spindle-ends appear to be devoid of centrioles. If the fixation is good, and suitable staining methods are adopted, there is no central granule in any of the asters; though artificial "centrioles" can be produced by overstaining with iron-haematoxylin, as at other stages. Their size can be varied at will, according to the degree to which the stain is extracted. After careful study of all my preparations I feel convinced that such "centrosomes" and "centrioles" are really deposits of stain at the point where the astral rays and spindle-fibres converge. At all events, if a centriole is really present, as a solid and separate body, it must be excessively minute; and the better the fixation and staining, the less often can any trace of it be seen[1].

During the whole of this first division period the karyosomes of the original macrogamete nucleus continue to undergo fragmentation (see Figs. 108, 115,

[1] There is certainly, as a rule, no large "centrosome," like that figured by Pixell-Goodrich (1914, Pl. XIII, fig. 10) at this stage. Such large bodies are, in my experience, only seen in imperfectly fixed preparations which have been stained by a watery iron-alum haematoxylin process (*e.g.* Heidenhain's).

121, etc.). Small fragments are scattered all through the cytoplasm, and many of them are often seen lying about the chromosome groups (cf. Fig. 121). Some of them may ultimately become incorporated in the daughter-nuclei (Figs. 125, 128); and I believe that some, at least, of the darkly staining bodies in the resting nuclei (Figs. 129–131) are derived from this source. From their general behaviour and unequal distribution, however, it is clear that they play no active part in the formation of the daughter-nuclei.

In small parasites, which form only 4–6 nuclei, the telophases occur in all nuclei synchronously. But in large individuals which form many daughter-nuclei, these often pass through their final stages independently. Some nuclei can be seen completely reconstructed and at rest while others are still undergoing or finishing division. The final stage is, however, always the same—a stage at which all the nuclei are in a completely resting state at the periphery of the organism (cf. Figs. 130, 131). This stage, from its frequency in preparations, is evidently one of long duration[1].

After remaining at rest for some time, the nuclei begin to divide again (Fig. 132), but this time by a simpler bipolar mitosis closely similar to that seen during the comparable stages in the male. A spireme is formed in each nucleus, and from it six filamentar chromosomes are formed. At the same time an achromatic body makes its appearance at the spot on the periphery of the nucleus where it was last seen (Fig. 128); and it gradually develops into a small projecting cone, which soon divides into two. The chromosomes arrange themselves in a bunch at the base of the cone, and when it divides they split longitudinally. The cones then draw apart, each of them taking a group of six filamentar chromosomes with it. Fig. 132 shows a number of stages in this process, and also illustrates the fact that division does not occur simultaneously in all the nuclei. Further details in similar nuclei at a slightly later stage, and at a higher magnification, are shown in Fig. 135 (division of achromatic cone, splitting of chromosomes), Fig. 136 (separation of cones and chromosome groups—early anaphase), and Fig. 137 (late anaphase group, attached to cone). The nuclear membrane is visible up to the time when the daughter-groups separate, but then disappears (cf. Figs. 132, 135, 136). Spindle-fibres are not, usually, distinctly visible between the chromosome groups; but the achromatic cones sometimes show a faint striation, and a few indistinct astral radiations into the surrounding cytoplasm. I have not been able to demonstrate a centriole at the apex of the achromatic cone.

This method of mitosis is exactly like that seen in the male at a similar stage: but it is a very remarkable mitosis, as there is no proper achromatic spindle—merely two spindle-ends, as it were—and no equatorial plate. The

[1] Siedlecki's account (1898) of the early nuclear divisions of the sporont is certainly incorrect in detail. He believed that the zygote nucleus divided by constriction into two, and that the daughter-nuclei then continued to divide in the same way many times in succession. His Fig. 22 (Pl. IX) shows, in reality, a stage similar to my Fig. 115 (Pl. IV), and not a stage in simple bipartition. The early nuclear divisions are very much more complicated than he supposed them to be.

chromosomes are filamentar throughout, and usually very difficult to count. As a rule, however, the two longest ones (*a* and *b*) can be readily distinguished —especially in the earlier stages (Fig. 135) and at the time when the groups are pulled apart in the late anaphases[1] (Fig. 137); and occasionally they can all be recognized with certainty, though they are always difficult to show in a drawing. During the later stages I have seldom been able to make out the smallest (*f*) chromosome with certainty, though I believe[2] it is always present.

The nuclei continue to divide in the same way—though not synchronously —on the surface of the organism, and as they do so they gradually become smaller. Resting nuclei, during the whole of this later development, stain deeply and easily, and never have the peculiar "achromatic" appearance of those formed at the first resting stage. As the nuclei increase in numbers, the surface of the organism gradually becomes more and more folded. These stages have already been figured by other workers, so I give only two illustrations of them—though they are always plentiful. Fig. 133 shows a surface view of a medium-sized sporont (whole) from the upper part of the intestine. Fig. 134 is a section of a very large specimen[3], at a later stage, from a lower region of the intestine. These figures will serve to illustrate the kind of folding that occurs. When the folding is complete, the organism resembles a very deeply furrowed and irregularly convoluted brain, whose whole surface is closely studded with small and slightly projecting nuclei.

As the folding proceeds, the organism becomes more and more separated from its capsule, or oocyst. This, as we have already seen, is formed by the original cellular investment of the parasite together with the fertilization membrane. At a stage such as that shown in Fig. 134, the oocyst is always well developed, and can be seen to consist of several layers (not shown in figure). The cyst is more or less spherical in the living organism, but becomes folded or distorted in fixed preparations (as in Fig. 134). In smears it can often be seen intact; but it may be torn or removed in making preparations, as it is adherent, externally, to the host's tissues. A clear liquid occupies the space between the protoplasm and the inner surface of the cyst.

The bigger the parasite, the more convoluted does its surface become: but although certain forms are commoner than others, the convolutions display no constant or regular pattern. Nuclear divisions continue all the time, and as the nuclei become smaller and more numerous they become more difficult to study. The divisions are always mitotic, however, and the chromosomes can occasionally be counted during this period: but the only chromosomes

[1] Such stages have already been figured by Siedlecki (1898, Pl. IX, fig. 24), who evidently saw the *a* chromosomes quite clearly, though he gives but a slight account (p. 830) of the mitotic divisions at these stages.

[2] Chiefly for the reason that it is present in earlier divisions, occasionally visible during those now under discussion, and again recognizable in the sporal divisions later. There is no reason to suppose that it ever drops out and is re-formed.

[3] Note that this figure is drawn under a much lower magnification than Fig. 133, and is therefore very much larger, in comparison, than it here appears.

which are, as a rule, clearly visible, are the two longest (*a* and *b*). Except that they are smaller and more difficult to study, the final nuclear divisions of the sporont do not differ in any notable way from those which occur in the earlier stages—immediately after the first resting period. They are closely similar also to those in the male immediately before gametogenesis.

It will be evident from the foregoing description that, after meiosis, the chromosomes are present in the same form and number at every nuclear division in the sporont. No reduction occurs during these stages—the haploid set of six chromosomes being present throughout.

(9) *Sporogenesis.*

When the superficial foldings and nuclear divisions of the sporont are completed, the nuclei all enter the resting state. They become spherical, somewhat coarsely granular vesicles, measuring as a rule about 3 μ in diameter, and are therefore larger than the nuclei of the male at the corresponding stage. They are also not so close together. At first they merely project above the surface; but very soon the cytoplasm becomes differentiated into numerous rounded masses, of uniform size, each containing a single nucleus. These masses are the sporoblasts, which, when fully formed, appear under a low magnification clustered together like bunches of grapes.

The formation of the sporoblasts is easy to observe—all stages occur frequently in preparations—and has been described many times. The sporoblasts first appear as mammiform processes on the surface of the sporont (Fig. 138), and each such process is finally constricted off at its base as an independent body (Fig. 139). In the formation of these bodies (sporoblasts) the whole of the cytoplasm of the sporont is used up, so that no protoplasmic residue (oocystic residuum) is left. Each mammiform process (Fig. 138) terminates in a pointed nipple-like structure, formed by the achromatic cone which has persisted from the last nuclear division. Immediately below the nipple lies the nucleus, which is coarsely granular and reticulate and often contains a deeply staining, elongate or comma-shaped karyosome (Fig. 138). When the sporoblasts are first formed they have a very characteristic shape—being not round but pear-shaped or lemon-shaped (Fig. 139), and appearing triangular rather than circular in outline. Each has a long pointed nipple, attached by a faintly fibrillar central structure to the nucleus. Whilst they are forming, a rounded or oval mass of reserve material[1] appears in each sporoblast, and gradually increases in size. Sometimes more than one such mass is present. Its chemical composition I have not been able to determine; but it is not composed of glycogen or chromatin, is insoluble in most reagents, and stains feebly with most stains (cf. Fig. 139). Apart from these masses,

[1] Previously described by Moroff (1908), p. 124. According to him the large mass is formed by the fusion of several smaller masses, and this interpretation appears plausible. He did not determine the chemical composition of the substance, but says that it is "sicherlich ein Umwandlungsprodukt des Chromatins"—which I question. He notes also that these reserve bodies do not occur in all species of *Aggregata*.

the cytoplasm shows no inclusions except occasionally a few minute bright granules.

The mammiform precursors of the sporoblasts, and the early piriform or "triangular" sporoblasts themselves, have been described and figured already by Moroff[1]: but for some unexplained reason he attributed them to two "new species" of *Aggregata*—not to *A. eberthi*, to which they undoubtedly belong. The number of sporoblasts formed depends upon the size of the sporont which forms them—large sporonts forming hundreds, smaller ones a few scores or dozens. But however many may be formed, the sporoblasts themselves are always of about the same size, and all ultimately produce identical spores in the same way. In every normal and well fixed specimen the sporoblasts show the characteristic forms just described. It is thus impossible to subdivide *A. eberthi* into a number of separate species—or even varieties—distinguishable by the form of their sporoblasts.

At first the sporoblasts are arranged in clusters, but they soon become uniformly distributed through the oocyst. At the same time they change their shape, and become spherical. Their nipples become smaller and finally disappear together with their contained achromatic structures; and soon afterwards each sporoblast secretes a gelatinous envelope round itself (Fig. 140). This investment—the first instalment of the spore wall (sporocyst)—is at first thin and uniform, but soon becomes irregular and thickened. It is later replaced by a much thicker and harder investment which is formed beneath it, closely adherent to the protoplasm (Pl. VI, fig. 141). The soft outer coat, first formed, then appears to become loose, and is ultimately shed.

The sporocyst is always formed in this way in *A. eberthi*, and the two spore-coats were first seen and described by Schneider (1883). He called the deciduous outer coat the "epispore," the permanent inner one the "endospore." With him, I believe that these two coats are observable in the spores of all Coccidia. In *Aggregata* they are very obvious, and can be clearly seen and studied. They differ conspicuously from one another in their staining reactions, and can be clearly displayed by various double-staining methods: for example by Mann's stain, which colours the epispore pale blue, the endospore bright pink[2]. When the spore is fully formed it is invested by the resistant endospore only—the epispore having been cast off during development.

Whilst the sporocyst is forming, the nucleus undergoes further changes. Each sporoblast is, normally, uninucleate. Its nucleus (Pl. V, fig. 140 and Pl. VI, fig. 141), measures about 3μ in diameter, and is finely granular, usually

[1] Moroff (1908), Pl. IX, fig. 86 ("*A. mamillana*") and Pl. XI, fig. 102 *a–c* ("*A. arcuata*").

[2] I add these details because various writers—for reasons incomprehensible to me—have disputed the accuracy of Schneider's observations. Labbé (1896, p. 607) makes the extraordinary mistake of supposing that the epispore forms the permanent wall—*i.e.* is not deciduous, as correctly described by Schneider—and that the endospore is a thinner and more delicate investment lying within it—*i.e.* in contact with the protoplasm. He appears to have misread Schneider's description, but to have applied his terminology to his own erroneous observations.

with a large peripheral karyosome[1] and an indistinct membrane. As the sporocyst develops[2], however, the nucleus becomes larger (Fig. 142), until it may attain a diameter of as much as 5·5 μ. It is then seen to be filled with evenly distributed chromatin granules on a linin network, and surrounded by an evident membrane. Its position is usually eccentric. From the network, by rearrangement of the granules, a spireme thread is now formed (Fig. 143), which opens out into loops and finally breaks into six free filamentar chromosomes (Fig. 144). In favourable specimens the typical complex *a–f* can now again be made out distinctly.

An achromatic spindle is now formed, the nuclear membrane vanishes, and the six chromosomes pass on to the equatorial plate. As they do so, they shorten and thicken—just as they do at the first division in the male and at the meiotic division—so that they finally appear on the equatorial plate as globules, not as filaments (Figs. 145–147). The equatorial plate, when seen from the side (Fig. 147), is difficult to study, for the chromosomes usually appear as an almost solid mass: but they can be counted clearly, and their typical size-relations made out with certainty, in favourable polar views. Fig. 147 shows the spindle in side view: Fig. 145 shows a polar view of the plate at the time when the chromosomes are changing from the filamentar to the globular form: Fig. 146 shows a chromosome group only, seen slightly obliquely, at the time when they are all more or less globular and massed on the equatorial plate. In all these figures the typical chromosome complex *a–f* is clearly visible[3].

At metaphase each of the six globular chromosomes divides by constriction into two equal parts. Fig. 148 shows a polar view of a plate at this stage. The smallest (*f*) chromosome is here completely divided, the others are in process of division. Whether the *f* chromosome always divides in advance of the rest—as it sometimes does at other divisions—I am unable to state. It certainly does sometimes, but it is usually, owing to its small size, very difficult to study.

The metaphase groups now separate and pass towards opposite poles of the spindle. As they do so they become drawn out into filaments once more

[1] Moroff (1908, p. 125) says that this body "plays the part of a centrosome" in the ensuing nuclear division: but this is certainly incorrect. It behaves like an ordinary karyosome, and its chromatin contributes to the formation of the chromosomes in the prophases of the first sporal division.

[2] It should be noted that in Figs. 142–159 the spore walls (sporocysts proper) are not usually depicted. Only the sporoplasm is drawn in most of them. Moreover, as these drawings are made from spores contained in sections, their diameters do not represent the true diameters of intact spores. The spore itself is a sphere, and the sporoplasm is a smaller sphere lying within it: consequently, the diameters of both spore and sporoplasm *in a section* will vary with the plane of section. In reality, all the stages figured are from spores of approximately the same size.

[3] Moroff (1908), who was the first observer to describe the mitotic divisions in the spore of *A. eberthi*, evidently never saw many of these stages, and his account is thus incorrect and incomplete. He says that at the first sporal division in "*A. mingazzini*" (really *A. eberthi*) he was "almost always" able to count eight chromosomes (Moroff, 1908, p. 126). He gives no figures showing this number, however, though he noted (p. 125) that "the chromosomes differ greatly in length"—some being short, others much longer.

(Figs. 149, 151), though in the early anaphases they still remain recognizable and countable in favourable specimens (Fig. 149). In the later anaphases, however, they assume figures (Fig. 151) which are very similar to those seen at the first division in the male (cf. Pl. II, fig. 52) and the first division of the sporont (cf. Pl. IV, fig. 114), and consequently it becomes impossible to enumerate them with certainty. The poles of the spindle draw further and further apart, and the daughter-groups finally separate (Fig. 152). The chromosomes shorten and thicken, a nuclear membrane is formed, and the telophases occur in the typical manner. After the chromosomes have broken down, and the nuclear membrane has been fully formed, the sporoplasm thus contains two resting nuclei (Fig. 153), which are usually oval and measure about $3\mu \times 2\mu$. The first sporal division is thus completed.

I have not been able to ascertain exactly how the achromatic spindle is formed. In the early stages it generally appears very indistinct in my preparations, and only becomes definite when the equatorial plate is fully formed (Fig. 147). At later stages (*e.g.* Fig. 149) it is sometimes very clear, and shows distinct spindle-fibres but no astral radiations. I have been unable to demonstrate centrioles at its poles. In the later anaphases the conical spindle-end (Figs. 150, 151) is seen attached to the chromosomes, which it accompanies into the resting nucleus; but at the final stage of rest (Fig. 153) I have been unable to find any trace of this structure. When the sporoblasts are first freed, an achromatic cone, derived from the previous mitosis, is present in the "nipple" (Fig. 139), as already noted. It is possible that it persists when the sporoblasts become spherical, and ultimately forms the spindle for the first sporal division: but I have not been able to trace the continuity of these structures.

The sporoplasm probably remains binucleate for a considerable time, for spores containing two resting nuclei (Fig. 153) are very common in preparations. At this stage the only other conspicuous structure in the spore is the large oval or round reserve body. After a time, however, one of the two nuclei again divides mitotically into two, so that the spore becomes trinucleate. This division is in every way like the first, except that it is on a smaller scale. A spireme is first formed, consisting of six loops (Fig. 154) which break apart into six filamentar chromosomes (*a–f*). An achromatic spindle is then formed, and the chromosomes shorten and thicken as they pass on to the equatorial plate (Fig. 155). On the plate (Fig. 156) they become globular again, and can be counted, and their size-relations can be observed. They then divide, as globules, by constriction into two at metaphase (Fig. 157). As the spindle-ends draw apart at anaphase, the chromosomes become filamentar, as before, and they finally separate (Fig. 158) and give rise to a pair of smaller resting nuclei during the telophases (Fig. 159)[1].

[1] According to Siedlecki (1898, p. 830) the three nuclei are formed by a simultaneous constriction, into three parts, of the originally single nucleus of the sporoblast. He describes the process as "a simple amitotic division." As will be evident from the foregoing account, the three nuclei are never formed in this way in *A. eberthi*.

Whilst this nuclear division has been taking place, the sister nucleus remains at rest (Figs. 154, 155, 158, 159); but it usually acquires a coarsely granular structure, and enlarges somewhat (cf. Figs. 155, 158 with 153, 154). When the second sporal division is completed, therefore, the sporoplasm contains three nuclei—one larger than the other two, which are of approximately equal size (Fig. 159). All the nuclei at this stage have a coarsely granular structure, appear more or less irregular in outline, and generally lie equidistant from one another. But gradually they become more regularly rounded, and finally all three acquire the same structure and size (about 2μ in diameter).

After remaining in this trinucleate condition for some time, the sporoplasm begins to undergo differentiation. An equatorial constriction appears, dividing it incompletely into two almost equal halves—one containing the three nuclei, the other containing the reserve body. At about the same time three meridional clefts appear, subdividing the nuclear half into three equal parts (Fig. 160). The sporoplasm now shrinks away from the sporocyst, and its cleavage continues until complete separation into four segments has taken place—one large segment containing the reserve mass but no nucleus, whilst the three equal smaller segments each contain one nucleus (Fig. 161). These nucleate segments are the young sporozoites[1]. When first cut off they are subconical, but they soon become sausage-shaped; and their nuclei, which are originally central, soon take up a terminal position (cf. Figs. 160 and 161). At this stage (Fig. 161) the sporozoites measure about 6μ in length, and their vesicular nuclei are almost spherical. Occasionally a minute spherical body can be seen at one pole of the nucleus (Fig. 162). It appears to be the remains of the achromatic cone, but I have not been able to trace its connexion with the achromatic structures present at the sporal divisions.

The sporozoites now grow in length, becoming long vermiform bodies which ultimately attain a length equal to nearly twice the diameter of the sporocyst which contains them. In the course of growth they become closely wound together in a spiral (Fig. 163): their nuclei also become more elongated and denser, but retain their terminal position. As the sporozoites grow, the large mass of protoplasm containing the reserve body (Fig. 161) gradually diminishes in size, until, at the completion of development, it is represented by one (or sometimes more than one) very small residual ball—the sporocystic residue—which is not absorbed (Fig. 163). It is clear that the sporozoites grow at the expense of the sporocystic residue; but how they are able to do so—being entirely separate from it—I do not know. In the living spore the residue appears as a mass of bright granules, and its exact relation to the sporozoites is difficult to make out.

When fully formed the sporozoites (Fig. 163) measure about 15–17 μ in

[1] The spore of *A. eberthi* figured by Siedlecki (1898, Pl. IX, fig. 26) is evidently an immature one at this stage—in polar view, as in my Fig. 162. Labbé's figure, on the other hand (1899, Fig. 108, p. 54), showing three long sporozoites with central nuclei, is unlike anything I have ever seen in *A. eberthi*.

length and 1·5μ in breadth. Their nuclei are massive and terminal, and measure 4–5μ in length. The sporozoites always lie in the sporocyst in the position shown in Fig. 163. Their nucleate ends are all at one pole, so that the spore, when seen from this end, always has the characteristic appearance shown in Fig. 164. The spiral arrangement, moreover, is invariably the same—always a *left-handed* spiral. Although I have examined a very large number of spores I have never found a single certain exception to this rule. This fact has not been noted before, I believe, but it seems to me noteworthy[1].

Fully-formed spores contain only a very small sporal residuum, and nothing else besides the three closely wound sporozoites. The number of these is very difficult to count in the fresh state, as the spiral into which each is wound consists of more than one complete turn.

The sporocyst, when examined fresh, appears nearly spherical: but after fixation it usually becomes slightly elliptical in outline (cf. Fig. 163). It consists—in the mature spore—of a single smooth, colourless, tough, and refractile membrane, about 0·5μ in thickness, and derived from the endospore. In stained spores (sections) the inner surface of the sporocyst often stains more intensely[2] than the rest (cf. Fig. 163); but apart from this there is no evidence that the spore is lined with a thin inner membrane (as alleged by Labbé). In reality the sporocyst is composed of two equal hemispherical valves, in close apposition. The suture between them can often be seen in crushed spores, and is sometimes visible in intact specimens after staining: but the valves are best demonstrated when the spore hatches. Ripe spores, as already noted, usually possess no epispore[3].

Spores within the same oocyst usually develop synchronously in the early stages, but afterwards they often develop at different rates. In the late stages they are generally all in approximately the same stage, but some are ahead of and others behind the majority. (For example, in oocysts containing spores mostly at the stage of the second sporal division, some may show the prophases of this division while others may already show three resting nuclei—with various intermediates.)

Mature normal spores usually measure 8–9μ in diameter in the fresh state, but some may be slightly larger and others slightly smaller. In fixed material their walls are frequently collapsed, and measurements are difficult to make: and in sections—as the cut often does not pass through the centre—they often appear to be considerably smaller than they really are. The spores are

[1] Moroff (1908), in his figures of the spores of *Aggregata* (more than one species) from *Octopus*, depicts an apparently similar left-handed spiral arrangement of the sporozoites. But so far as I can discover he nowhere mentions that the spiral is always—or even usually—in this form. Schneider (1883, Pl. IX, fig. 29) figures four gigantic sporozoites escaping from a diminutive sporocyst: and two of the four are depicted as right-handed spirals. Most other observers seem to have ignored the point altogether.

[2] Noted by Léger and Duboscq (1908, p. 58), but only in hatched spores.

[3] As correctly stated by Léger and Duboscq (1908, p. 58)—who add, however, that they have not observed the epispore in the earlier stages either.

of the same size in oocysts of all sizes. They vary greatly in numbers, but not in dimensions.

A. eberthi forms atypical spores with such frequency that the more usual abnormalities must be mentioned[1]. Such spores differ from the normal in their size and in the number of sporozoites which they contain. I have been able to follow their formation in detail, but have not space to figure all the stages.

The commonest abnormality is a spore containing four sporozoites instead of three (Fig. 165). Such a hyperzoic spore is formed exactly like a normal spore, with the exception that both nuclei divide at the second sporal division (instead of only one, as usually happens). The sporoplasm thus becomes quadrinucleate, and four sporozoites are then formed in the usual way. These tetrazoic spores are of the same size as normal trizoic specimens, but contain slightly smaller sporozoites. Spores containing six sporozoites are also sometimes seen (Fig. 166). These are larger (about 12μ in diameter), and are formed in a different way. They develop from an originally binucleate sporoblast, which is formed at the stage of segmentation (Figs. 138, 139) by two nuclei getting cut off in the same segment of cytoplasm. A large binucleate sporoblast is thus formed. It secretes its sporocyst in the usual way, and each nucleus divides in the usual way to form three daughter-nuclei—six in all. Six sporozoites of normal size are then formed within the spore. More rarely octozoic spores are formed from such double sporoblasts. In these each nucleus divides twice in succession, forming eight daughter-nuclei, and eight sporozoites of normal size result.

It is clear that the normal trizoic spore results from the inhibition of one sporal division—a division which actually occurs in the formation of (abnormal) tetrazoic spores. The hexazoic spore is evidently a double trizoic spore, and the octozoic a double tetrazoic.

Spores containing five, seven, and more than eight sporozoites are also very rarely formed by *A. eberthi*. A pentazoic spore begins like a hexazoic; but one of the nuclei in the (double) sporoblast divides only once, giving two nuclei, while the other forms three in the usual way—five nuclei and five sporozoites thus resulting. Similarly, a heptazoic spore begins like an octozoic; but one of its two original nuclei gives rise to four daughters, the other to only three. Such a spore is therefore a double spore, formed by the union of a trizoic with a tetrazoic.

I have very rarely found giant spores containing 10, 12, and 16[2] nuclei (or sporozoites). It is probable that the 16-zoic and 12-zoic spores are double octozoic and double hexazoic spores respectively: but I have not found all the stages in the development of spores containing more than eight sporozoites.

[1] I shall say nothing, however, regarding abnormalities—which are not at all uncommon—due to degeneration and faulty technique. But I may remark that the spores of *Aggregata*, in spite of their thin walls, are difficult objects to fix and stain properly; and that, even in the best material, the majority of spores are in consequence usually unfit for detailed cytological study.

[2] In spores containing more than ten sporozoites the counts are somewhat uncertain.

In all these hyperzoic spores the sporozoites are of approximately the same size, and appear to be normal in every way. So far as I have been able to determine, the nuclei divide in the normal way, and the typical set of six chromosomes is present throughout. There is no reason to suppose, therefore, that hyperzoic spores are incapable of further development in the crab: but I have no direct evidence on this point.

Occasionally cysts are found which appear to be oocysts containing a large number of free sporozoites—no trace of sporocysts being visible. The origin of these cysts long perplexed me, as I was unable to find any stages in their development. But I am now satisfied that the "sporozoites" are not really sporozoites at all, but peculiarly degenerated microgametes; and that the cysts are not oocysts, therefore, but the cysts of male parasites[1]. I have no evidence to show that sporozoites are ever formed freely—without sporocysts—in the oocysts of *A. eberthi*.

That spores containing more than three sporozoites belong to *A. eberthi*—not to some other species—and that they are abnormal, I conclude for the following reasons: (1) the vast majority of spores in every oocyst, irrespective of its size, are trizoic; (2) when hyperzoic spores occur, they are always isolated specimens lying among normal trizoic spores. Oocysts are never seen in which all the spores are, for example, tetrazoic or hexazoic; (3) from the development of hyperzoic spores, their relation to normal trizoic forms is at once apparent, and the production of each abnormality can be demonstrated at every stage; (4) the sporozoites in all hyperzoic spores are exactly equivalent in every way—including their chromosomic constitution—to those in normal trizoic spores.

I have devoted special attention to the development of the spores in *A. eberthi* because these stages are important, and neither in this species nor in any other coccidian have the sporal nuclear divisions been accurately described hitherto. Nearly all previous workers, it is true, have described and figured—more or less carefully—the spores of *A. eberthi*[2]; but not one of them has studied the nuclear divisions in the necessary detail. We now know, from the observations of Jameson (1920)[3], that the first sporal division in *Diplocystis schneideri*—and therefore probably in other Gregarines—is a reduction division: consequently, the sporal divisions of Gregarines and Coccidia generally demand further notice than they have hitherto received. I shall say no more on this subject here, but will conclude this section by emphasizing the following points in my observations on sporogenesis in *A. eberthi*: (1) all nuclear divisions immediately preceding and during spore-formation are mitotic; (2) the same number of chromosomes is present throughout; (3) this number is six, and is the haploid number; (4) no reduction occurs during either of the sporal

[1] It seems to me probable that similar abnormal specimens led Mingazzini to believe that the microgametes of *A. eberthi* were merozoites, and for this reason I mention them here.

[2] As all these descriptions are incomplete, and most of them faulty in details, it seems to me unnecessary to discuss them all here.

[3] First announced by Dobell and Jameson (1915).

divisions; (5) consequently, all sporozoites are equivalent in nuclear constitution—each containing the typical set of six haploid chromosomes.

(10) *The Earliest Stages in* Portunus depurator.

Ripe spores of *A. eberthi*, when discharged from the intestine of *Sepia*, are incapable of further development unless they are ingested by a suitable crab (in my experiments, *Portunus depurator*). The whole development (asexual cycle) in the crustacean host has already been described by Léger and Duboscq (1908), whose account I have been able to confirm in its essential points[1]. I shall therefore describe these stages very briefly—making such additions as I can to their account.

As described by Léger and Duboscq, the spores liberate their sporozoites in the crab's gut (Pl. VI, fig. 167). Each sporocyst is then clearly seen to be formed from two closely sutured hemispherical valves, which appear to be somewhat elastic: for when they separate[2], they become longer and shallower (Fig. 167). The sporozoites which emerge (normally three from each sporocyst) are active, vermiform organisms measuring about 16–17 μ in length when extended, but capable of contraction to a length which is several microns less. Their width is 1·5 μ to about 2 μ—according as they are extended or contracted. The nucleus is elongate, compact, deeply stainable, and terminal—as in the fully-formed sporozoite within the spore (cf. Fig. 163). The nucleate end of the sporozoite is posterior, the opposite more pointed end anterior. A few residual granules are left in the sporocyst when the sporozoites escape.

The sporozoites move actively in the gut contents, and soon penetrate the epithelium lining the midgut (Figs. 168, 169). They pass through this rapidly, and then encounter the basal membrane. Normally, the sporozoites are able to bore their way through this also (Figs. 170 and 171 show two stages in the process): but when the basal membrane is very thick, it may arrest their passage more or less completely (cf. Fig. 169). After traversing the inner layers of the gut wall, the sporozoites enter the submucous connective tissue, where they come to rest and begin to grow into schizonts. All the stages just mentioned have already been adequately described by Léger and Duboscq. I will only add that they are closely similar to the earliest stages seen in the cuttlefish—the merozoites and sporozoites behaving in exactly the same way.

Léger and Duboscq say little about the precise habitat of the parasite in the submucous tissue: but from their statement[3] that the organisms "never enter any of the haemocoelic spaces except the circumintestinal sinus," I conclude that they suppose them to lie ultimately in the lymph-spaces in the connective tissue—not intracellularly. There can be no doubt, however, that the sporozoites sometimes enter and grow into schizonts inside the connective-

[1] See subsection (A) of the present section, *supra*.

[2] The valves usually remain attached at one point (cf. Pl. VI, fig. 167, and Léger and Duboscq's (1908) Figs. 2 and 3, Pl. V): and opened sporocysts thus resemble empty oyster-shells.

[3] Léger and Duboscq (1908), p. 60.

tissue cells (see Fig. 175). According to my observations they may develop either intercellularly or intracellularly—just as the merozoites do in *Sepia*.

Differentiation of the nucleus in the young parasites has been well described and figured by Léger and Duboscq (1908), so I can deal with these stages briefly. But I would lay stress on the circumstance that the early nuclear development is exactly like that which I have previously described in the young sexual forms.

The nucleus, at first dense and almost homogeneous (Fig. 167), passes from the end to the middle of the organism (Figs. 172, 168, 173); and as it does so it becomes reticulate and granular. Two structures—the karyosome and the micronucleus—appear in it at a very early age (Figs. 172, 173), exactly as they do in the young sexual forms. The karyosome, at first homogeneous (Fig. 172), becomes hollow (Figs. 173, 174), and acquires a micropyle, over which the small deeply staining micronucleus takes up its position (Figs. 173–175). The micronucleus enlarges slightly, and finally passes through the micropyle (Figs. 174–177) and dilates within the karyosome into a very definite body with a vesicular structure and a reticulate arrangement of chromatin (Figs. 176, 177). All this occurs exactly as it does in the sexual forms. The peripheral chromatin grains also behave in the same manner—becoming less stainable and gradually disappearing as the nucleus grows (Figs. 173–177).

When the nuclear system of the young schizont is fully differentiated, it is therefore exactly like that of a young male or female (cf. Pl. I, fig. 13, and Pl. II, fig. 38, with Pl. VI, fig. 177). The nucleus is limited by a definite external membrane, and contains a homogeneous non-chromatic sap. Its single large karyosome consists of two layers—a deeply stainable cortex (sometimes alveolate or vacuolate, as in Fig. 178), and a less stainable medulla, in which the delicate vesicular micronucleus lies.

Léger and Duboscq (1908) saw and accurately described and figured all the stages in the formation of the karyosome and the micronucleus, the entry of the latter through the micropyle[1], and its subsequent growth into a vesicular nucleus-like body: but they called the karyosome a "nucleolus" and the micronucleus a "karyosomic body" (*nucléole* and *corps karyosomien* respectively). They regarded the former as composed of plastin, the latter of chromatin: but if I understand them aright, they believe that the micropyle is not present before the entry of the micronucleus, but formed by it as it passes into the karyosome. I have no doubt, however, that the account which I have given is essentially correct; though I am not prepared rigidly to define "nucleolus," "plastin," or "chromatin." I attach little value to these terms. In my opinion it is the morphological evidence that is really important in such matters.

[1] In exceptional cases Léger and Duboscq (1908) observed the formation and entry into the karyosome of two micronuclei. I have already noted that the same phenomenon occurs sometimes in young sexual forms, but I have not observed it in the schizonts. The phenomenon is doubtless abnormal in both cases, but its occurrence emphasizes the close resemblance observable between the young asexual and sexual forms in their nuclear structure and development.

(11) *Development of the Asexual Parasites.*

During the stages just described the parasites continue to grow. They ultimately become large, rounded, bulky schizonts, which may attain a diameter of as much as 150–200 μ. Their size depends, to some extent, upon the intensity of the infection—crowded parasites being smaller than those which are isolated. The cytoplasm of the schizont soon becomes filled with spherules—paramylum, according to Léger and Duboscq—and other inclusions, which give it a characteristic appearance.

As all these stages have been well described and illustrated by Léger and Duboscq (1908) I need say no more about them here. My observations agree, in general, with theirs—with the exception of one important point[1] about which I am still in doubt. According to Léger and Duboscq the schizonts are of two sorts, which they designate—with some hesitation—"male" and "female." Apart from the obvious impropriety of applying such terms to asexual organisms, I am doubtful whether any dimorphism really exists in the schizonts of *A. eberthi*. The French authors say that the two "sexes" can be distinguished by their size, rate of growth, cytoplasmic inclusions, and the thickness of their surrounding membranes. "The male...is always smaller, and differs from the female in its thicker membrane, in its alveolar network with smaller meshes, in its greater abundance of chromidial granules, especially at the periphery, and lastly in the smaller size of its paramylum spherules[2]."

I can confirm many of the observations on which these statements are founded. Some schizonts are smaller than others, some contain smaller paramylum spherules and more numerous "chromidial" and other inclusions, and some have much thicker membranous investments. Nevertheless, I have been unable to convince myself that the schizonts are dimorphic in respect of these characters. I have found so many intermediates between the extremes that I have frequently been unable to decide whether a given individual is a "male" or a "female"; and I think it most probable that these differences are merely individual variations dependent upon environment, state of nutrition, or other factors.

Though I am unable to distinguish two categories of schizonts with certainty, I am also unable to say that the conclusion of Léger and Duboscq is wrong. But I would point out that, if two different kinds of schizonts really do occur, then: (1) they are by no means easy to distinguish; (2) they cannot properly be called "male" and "female"—since both are asexual; and (3) the condition is almost unique, for although a similar dimorphism has been described in other Coccidia (*e.g. Adelea*), nearly every such instance has subsequently been found to be based upon error (*e.g.* in *Adelea ovata* the "two kinds of schizonts" really belong to different species[3]).

[1] And in respect of certain minor details of development already noted in section (A) above.

[2] Léger and Duboscq (1908), p. 64.

[3] Cf. Schellack and Reichenow (1915).

(12) *Schizogony*

The schizogony of *A. eberthi* has already been described and figured in detail by Léger and Duboscq (1908). According to their observations the nucleus of the schizont divides many times in succession by a peculiar mitosis, and a very large number of daughter-nuclei finally result. These distribute themselves all over the periphery of the organism, which forms a cyst wall and undergoes much superficial folding similar to that seen in the sporont. Finally each nucleus, with a portion of the peripheral cytoplasm, enters into the formation of a merozoite. When the merozoites are formed, the cysts have a characteristic appearance (Fig. 199), which recalls that of malarial oocysts on the stomach of a mosquito. No further development occurs in the crab—the ripe cysts remaining intact in the coelome of *Portunus* until it is eaten by a *Sepia*.

Although the account given by Léger and Duboscq is excellent, and I have been able to confirm it in most particulars, there are two important omissions from it. They were unable to study all the stages in the first mitosis: and they did not count, with certainty, the chromosomes present at any of the nuclear divisions[1]. Unfortunately, I too have failed to find all the stages of the first nuclear division; but I have, on the other hand, succeeded in counting the chromosomes present throughout the development of the schizont.

The prophases of the first nuclear division appear to me to occur precisely as they do in the corresponding stages in the male and the sporont. The micronucleus, after increasing in size (cf. Fig. 178), persists for some time inside the karyosome: and the structure of the latter is closely similar to that which I have described in the sexual forms. It also "buds" in a similar manner[2], so that a number of small accessory karyosomes are often present in addition to the main one. But usually the main karyosome is relatively larger in the schizonts—the daughter-karyosomes often being very small: and owing to its great size and density it is usually more difficult to demonstrate the micronucleus within it and to follow its development.

After a time the micronucleus disappears, and the medulla of the karyosome becomes filled with deeply stainable granules (Fig. 179). These are then discharged—chiefly through the micropyle—into the nuclear space. A net-

[1] In a footnote (p. 84) Léger and Duboscq (1908) remark that they believe the number of chromosomes present in the mitoses in the schizont to be five. But they clearly distinguished the *a* chromosome ("axial chromosome"); and their counts were probably correct, save only that they overlooked the very small *f* chromosome—which is frequently indistinguishable. I also should probably have failed to find it if I had not previously seen it at stages where it can be more clearly observed in the nuclear divisions of the sexual parasites, sporonts, and spores.

[2] Léger and Duboscq (1908) apparently believe—though they express themselves in different words—that the accessory karyosomes are, in part, constricted off from the cortex of the main karyosome, and partly formed by excretion through the micropyle. I would refer the reader to what I have already written about the "budding" of the karyosome in the sexual forms, which is equally applicable to the schizonts, according to my observations—so far as they go. (I have studied far more material containing the sexual than the asexual forms.)

work then forms—at first faint, then distinct—and resolves itself into a spireme, which then breaks up into a number of filamentar chromosomes. All these stages are described and illustrated by Léger and Duboscq, to whose account I can add nothing. All the stages which I possess are closely similar to corresponding stages in the sexual parasites, but my series in the schizont is incomplete. Judging from a single figure given by Léger and Duboscq (1908, Pl. V, fig. 21) I infer that the number of chromosomes formed from the spireme is probably six—as in the male and female at corresponding stages: but I have not, in my preparations, a single specimen in which I can count the chromosomes at this stage.

Although, for lack of material, I have not been able to study the process in such detail as in the sexual forms, I believe—from the similarity of all stages observed—that the chromosomes are formed here also from the substance of the micronucleus. Léger and Duboscq describe this organ as filling the karyosome so completely that it forms "un véritable noyau intranucléolaire qui constituera la couche médullaire du nucléole." I do not believe that the medulla of the karyosome is formed from the micronucleus, but that the two structures are distinct (cf. Fig. 178): but the medulla soon becomes so filled with stainable granules that the relations of the two are extremely difficult to make out. The description of the formation of the chromosomes given by Léger and Duboscq is not free from difficulties; but nevertheless, I feel justified in concluding—from their observations and my own—that the chromosomes are formed in the schizont exactly as they are in the sexual parasites.

All the stages in the formation of the first spindle in the schizont are, unfortunately, absent from my material[1]: and unfortunately also the description of these stages by Léger and Duboscq is incomplete, and their figures do not form a consistent series. I have little doubt that their account is not wholly correct, and that study of a larger series of stages will ultimately show that the behaviour of the chromosomes is closely similar to that which I have already described in the male. I shall therefore say nothing more on this subject here[2].

The first division of the schizont is a polymitosis like that already described in large sporonts: and after a variable number of rapid divisions have taken

[1] These stages, like the corresponding ones in the sexual forms, are evidently very difficult to obtain. The nuclei of the schizonts, on account of their large size, are also very difficult to study. In this respect they resemble the large female forms, in which I should never have succeeded in studying the chromosomes satisfactorily if I had not previously been able to make out the corresponding stages in smaller forms.

[2] I ought to add, however, that Léger and Duboscq (1908) believe that the chromosomes, after they are fully differentiated, do not pass on to the spindle, but form a new resting nucleus at the periphery: and from this they are re-formed, for the first mitosis, at a later stage. I hesitate to accept this account because it is not supported by figures of all the stages, and I cannot interpret some of the figures which are intended to illustrate the process. Their only figure showing the "reconstituted nucleus" (Pl. V, fig. 23) is exactly like an abnormality which occurs occasionally in the sexual forms. I know, in this case, that it is not a stage in normal development, and I believe, therefore, that Léger and Duboscq were mistaken in their interpretation.

place—without any resting periods intervening—the daughter-nuclei enter a resting state at the periphery. Thereafter, also, they divide by the simplified bipolar mitosis which I have already described in the later divisions in the male and in the sporont. My efforts have been chiefly directed towards studying and counting the chromosomes during these stages.

In my preparations the earliest stage in the first mitosis is the early anaphase shown in Fig. 180. The chromosomes are not clearly countable here, but there are approximately six—certainly not 12—in each daughter-group. The figure is very like those which I have many times seen at a corresponding stage in the male (cf. Pl. II, fig. 50) and in the sporont (cf. Pl. IV, fig. 114): but whether it is also preceded by an equatorial plate stage with globular chromosomes I am unable to say. During the polymitosis which follows, the chromosome groups are usually much flattened out on the surface of the schizont (Fig. 181), and the chromosomes—owing to this, and also to the splitting which they undergo—are here uncountable, though the two longest (*a* and *b*) are often recognizable. At the later stages in this division, however, I have been able to count the chromosomes with some certainty, as they emerge to form the resting nuclei. One such stage is shown in Fig. 184. The whole haploid complex (*a–f*) is here visible and every member can be recognized. All of them—including the *f* chromosome—can also sometimes be clearly made out in polar views of such late anaphase groups (Fig. 183); and again later, when the chromosomes shorten and thicken before the telophases (Fig. 182).

It is thus clear that the first nuclear division of the schizont—the polymitosis—gives rise to a number of daughter-nuclei each of which contains the typical haploid set of chromosomes (*a–f*). Moreover, since the schizont is itself merely a grown-up sporozoite, and since we know that the nucleus of each sporozoite receives a haploid set of six chromosomes during the sporal divisions, it must be concluded that this haploid set is present originally and occurs at every nuclear division in the schizont up to the time when the resting nuclei are formed after the polymitosis.

During the subsequent bipolar mitoses the chromosomes are again very difficult to count. The mitoses are like those in the male or the sporont (after the polymitosis), and the chromosomes are filamentar throughout, while no equatorial plate is formed. In the earlier divisions they are fairly large, and can sometimes be counted with fair certainty during the late anaphases. Later, as they become smaller and more compact, the nuclear figures are very difficult to study. Figs. 185 to 193 are drawings of chromosome groups during these divisions. In a few (*e.g.* Figs. 185, 186, and 192) all six chromosomes are recognizable: but in figures such as 190, 191, and 193 only the *a* and *b* chromosomes are clearly distinguishable—owing to their greater length.

I have no doubt that the chromosomes are present in their typical form and number (six) throughout all these divisions, though it is frequently impossible to recognize and count them with certainty—as in the corresponding

stages in the male and in the sporont. It is certain, in any case, that the number is not halved during these divisions, and at least two individuals (*a* and *b*) of the haploid group are distinguishable throughout. Moreover, as just noted, the haploid group is demonstrable in the earlier schizogonic divisions, and occurs again in the earliest stages of the sexual parasites which develop from the merozoites. It is thus highly probable that the chromosomes retain their individuality, and remain unchanged in number, during all the nuclear divisions of the schizont. The figures of Léger and Duboscq, also, though they are not conclusive in this respect, agree in general with my findings[1].

In some of the earlier divisions in the schizont the achromatic cone has a minute but definitely differentiated apical knob, like a centrosome (Figs. 183, 184). Such a structure is not usually demonstrable at other stages.

When the nuclei have finished dividing, and enter into the resting stage at the periphery, they are small, coarsely granular, and compact (Fig. 194). They measure about 2μ in diameter at this stage, and are approximately spherical. The formation of the merozoites has been described accurately by Léger and Duboscq (1908), so I give only a few figures illustrating their differentiation (Figs. 194 to 198). In the early stages a minute centriole-like body can often be observed near the outer pole of the nucleus, and often attached to it by a short stalk (Figs. 194, 195). Though I have devoted considerable attention to it, I am unable to say how it is formed, or whether it is really a centriole. In some individuals (*e.g.* the middle one in Fig. 195) it appears to be dividing after separating from the nucleus: but later the merozoites contain a number of stainable granules (Fig. 196), and I have been unable to distinguish the "centriole" among these.

As the merozoites grow in length, they become pointed at their distal ends (Figs. 195–197), and their nuclei become elongate and pass to the proximal extremity. Fully formed merozoites show the structure shown in Fig. 198. Each has an elongate reticular nucleus, terminal in position, and several cytoplasmic structures which are difficult to interpret. Immediately in front of the nucleus there is a well-defined clear area—representing an inclusion or vacuole—and in front of this a fine central filamentar or rod-like body[2] extends almost to the anterior extremity, which is furnished with a knob, or mucron. Whether the axial filament is formed from the centriole or not, I cannot make out with certainty. It is present in every fully-formed merozoite, and is probably skeletal in function.

All the stages just noted have already been described by Léger and Duboscq (1908). These authors, however, describe the merozoites as dis-

[1] Cf. Léger and Duboscq (1908), Pl. VI. Their Fig. 29 depicts a section in which the chromosomes are evidently cut, and incompletely shown. Their Fig. 34 shows the *a* and *b* chromosomes clearly, and possibly the whole haploid complex is visible in the actual specimen from which it was drawn. (It can, indeed, be made out—with a little imagination—in the figure.)

[2] A similar filament has been described in the merozoites of the peculiar sporozoon *Selysina* by Duboscq (1918). In the same paper he also gives two original figures (Fig. XI, p. 31) of the merozoites of *A. eberthi*.

tinguishable into two categories—those formed from "male" and "female" schizonts respectively. I have been unable to make out any differences of this sort in my material. All merozoites appear to me to be alike, and to develop in precisely the same way.

Fully-formed merozoites (Fig. 198) are usually shaped like bananas, and are at first attached radially to the large residual masses of the schizont (Figs. 197, 199). But later they break loose, and become free inside the cyst—floating among the disintegrating remains of their parent. They measure about 10μ in length and 2μ in breadth at the broadest part. Their nuclei are about $3{\cdot}5\mu$ long and contain no definitely individualized karyosomes or other structures, being uniformly granular and reticulate.

The merozoites undergo no further change until they are swallowed by a cuttlefish, whereupon they begin to develop into sexual forms in the manner described in earlier pages.

V. SYSTEMATIC. NOMENCLATURE

In the preceding pages I have described the development of *A. eberthi* in some detail. If the reader accepts this account he will already be convinced, I think, that *Aggregata* belongs to the Coccidia[1] and not to the Gregarinida. This conclusion appears so obvious, now that the whole life-cycle is known, that it seems to me to call for no further discussion. Moroff alone, among modern workers, has tried to show that *Aggregata* is a Gregarine: but his evidence, on his own admission, was inconclusive, and may now be regarded as definitely refuted. The proposal—made by Brasil (1907) and Léger and Duboscq (1908), and adopted by Fantham (1908)[2]—to put *Aggregata* among the Schizogregarines, no longer calls for discussion. Now that a detailed study of the chromosomes has been made, and the meiotic phases have been discovered, the problem of the systematic status of *Aggregata* has ceased to exist. There is no longer any reason to question Siedlecki's interpretation of the cycle in the cuttlefish; and consequently, as Léger and Duboscq (1915) have remarked, "la nature coccidienne [des *Aggregata*] n'est plus douteuse."

I have already pointed out repeatedly that all the different forms of *Aggregata* found in *Sepia officinalis* belong to the same species; but a few more words on this subject are necessary. It will be recalled that Moroff (1908) distinguishes at least six different species of *Aggregata* in *Sepia*. Not one of these is adequately characterized, however, and some of them—as is evident from his accounts—he cannot himself recognize at all stages with certainty. As I have already shown, there is great variation in size in *A. eberthi*, and the

[1] Notwithstanding its alternation of hosts, *Aggregata* is, in my opinion, a typical member of the Coccidia. When other members of the group have been studied in as much detail, Reichenow's doubts on this score will, I think, probably be removed. There is at present little justification for supposing that the Aggregatidae are atypical forms. They have two hosts—like the Haemogregarines—but apart from this peculiarity of habitat they appear to be in no way aberrant.

[2] I may note that in his review of this paper in *Bull. Inst. Pasteur*, VII. 394, Mesnil already at that date (1909) definitely rejected the gregarine interpretation of *Aggregata*.

form of the parasite at certain stages is dependent upon its magnitude—large sporonts and males, for example, undergoing more superficial folding and producing a greater abundance of spores or microgametes. That all forms belong to one species appears to me certain from the following considerations—among others:

(1) Every intermediate between the various forms may be found. If Moroff's attitude were adopted, therefore, and each form regarded as a distinct species, there would be not merely six species of *Aggregata* in *Sepia* but 60 or 600—according to fancy.

(2) All forms of parasite—whether large or small—normally produce spores of the same size and containing the same number of sporozoites. (Variations in these respects are observable equally in all forms.)

(3) The sporozoites are normally of the same size and form in the sporocysts derived from all forms.

(4) The microgametes, though varying in numbers from cyst to cyst, are identical in size and structure, and develop in the same way from parasites of all sizes.

(5) The nuclear development—when allowance is made for variations dependent upon the size of the organism—is identical in all forms.

(6) The chromosomes are the same, both in form and in number, in all parasites.

(7) All spores, from whatever form of parasite derived, appear to undergo exactly the same development in *Portunus*, in which they produce identical infections[1].

(8) No specific differences are observable in the schizonts obtained experimentally in crabs; and all probably have the same number of chromosomes—that found in the sexual forms—at every stage.

All Moroff's species from *Sepia* are said to have a "round karyosome." They cannot, therefore, be discriminated by this character. *A. eberthi*, *A. arcuata*[2], and *A. minima* are said to have trizoic spores, and are thus not separable by this feature: but the diameters of their sporocysts are given, respectively, as 8–19 μ, 6–8 μ, and 5–7 μ. As these measurements overlap, this character is also unsatisfactory. (A trizoic spore of 6–7 μ, for instance, might equally well be referred to *A. arcuata* or *A. minima*.) *A. mingazzinii*[3] and *A. mamillana* are said, however, to possess tetrazoic spores: but such spores are frequently found in *A. eberthi*. Moreover, as they invariably occur in oocysts in which the majority of the spores are trizoic and otherwise typical, it is impossible to attribute them to a different species. The sizes of all Moroff's species also overlap to such an extent that this again is a character

[1] There is perhaps some doubt about this. If the spores from *Sepia* be supposed to represent a mixture of species, then the evidence might be interpreted as showing that only one of these is capable of further development in *Portunus*.

[2] This species is first announced (Moroff, 1908, p. 103) as "*A. arculata*"—presumably a misprint.

[3] Also often referred to by the author as "*A. mingazzini*"—presumably owing to misprints.

which can hardly be used for systematic purposes. *A. arcuata* is further characterized by the arched shape of its sporont (in section) at a certain stage of folding: but this and many other types of folding may be seen in *A. eberthi*, and the amount of folding and its resultant pattern depend upon size and stage of development, and are worthless as systematic characters. *A. frenzeli* is not defined at all—unless "Parasite 80–100 μ. Karyosome round. Sporocyst?" and the information that it occurs in the caecum of *Sepia*, can be called a definition. There is nothing here to justify a separation from *A. eberthi*. Finally, *A. mamillana* is distinguished by the mammiform appearance of its sporoblasts at the time of their formation: but this, according to my observations, is the normal appearance at this stage in all "species" in *Sepia*.

It is hardly necessary to discuss further details. What I have said will suffice to show, I hope, that Moroff's system of classification is unsound. His six species of *Aggregata* from *Sepia* appear to be merely various forms or stages—some of them abnormal—of *A. eberthi*; and unless he can supply more cogent evidence[1] for their separate existence, in my opinion they ought all—except, of course, *A. eberthi*—to be invalidated.

The Aggregatidae appear to form a well characterized family of the Coccidia, and probably other genera besides *Aggregata* will ultimately be definitely assigned to it. It can hardly be doubted that *Merocystis*—inadequately described originally by Dakin (1911)—belongs to this family. The recent observations of Foulon (1919) seem to show this convincingly, but the life-cycle is still only partially known[2]. Another coccidian which may possibly belong to the Aggregatidae is *Orcheocystis* (Trinci, 1916); but the life-history of this organism is also incomplete[3]. Possibly *Pseudoklossia*[4], *Myriospora*[5], *Angeiocystis*[6], and other known genera may also be referable to the family, but I shall not attempt to discuss this question here.

As regards the position of the Aggregatidae among the Coccidia themselves, I will only remark that the family may be conveniently placed for the present in the position provisionally assigned to it by Léger (1911, p. 81)—as the fourth family of Polyzoica in his section Eimeriidea—or in the corresponding

[1] I ought to add that Moroff (1908, p. 143) himself regarded his classification as "provisional," and promised to treat the matter more at large in a later paper. As far as I have been able to ascertain, this later work has never yet been published.

[2] *Merocystis kathae* undergoes its sexual cycle (similar to that of *A. eberthi* in *Sepia*) in the renal organ of the whelk (*Buccinum undatum*). Its schizogony probably occurs in another host, but has not yet been discovered.

[3] *Orcheocystis lacertae* is so far known only from its schizogonic development (like that of *A. eberthi* in *Portunus*) in the testis of a lizard. Its sexual cycle should probably be sought in some other host.

[4] *Pseudoklossia* is known only from its sexual cycle and sporogony, which occur in the renal organs of Lamellibranchs (*Tapes*, *Pecten*). See Léger and Duboscq (1915, 1917), Debaisieux (1922).

[5] *Myriospora* undergoes its sporogony—forming spores very like those of *Aggregata*—in a marine polychaete (*Trophonia*). Its schizogony is unknown. See Lermantoff (1913).

[6] *Angeiocystis* occurs in the polychaete *Audouinia*, and is also known only by its sporogonic cycle. See Brasil (1909). It seems probable that both *Myriospora* and *Angeiocystis* undergo their schizogony in other hosts.

position in the modification of Léger's system proposed by Ikeda (1914). Reichenow's system (1920*a*, pp. 1184–5), in which *Aggregata* is placed in the family Caryotrophidae, while a new family (*Merocystidae*) is created for *Merocystis*, appears to me very artificial: but as I gather, from his own remarks on this system, that he does not advocate it seriously himself, I need not discuss it further here. Obviously the time is not yet ripe for a final classification of the Coccidia; but I may add that Reichenow's new system (1920*a*, p. 1186), in which they are divided into six families, seems to me to be a step in the right direction.

The nomenclature of *Aggregata eberthi* has been so often discussed that it is hardly necessary to go into it in detail again. I have read, I believe, everything of any importance that has previously been written on the subject, and my conclusions are summarized in the following list of synonyms. This, taken in conjunction with the references at the end of this memoir and with what has been said in earlier pages, will, I hope, explain itself without further discussion.

Aggregata eberthi (Labbé, 1896) Léger & Duboscq, 1906.

Synonyms:

"Einzelne kuglige Gregarinen" Lieberkühn, 1854.
"Grégarines" Lieberkühn, 1854*a*.
"Psorospermienschläuche" Eberth, 1862.
"Sac à Psorospermies" P. J. Van Beneden, 1875.
Klossia octopiana Aimé Schneider, 1883 (pro parte = *Benedenia octopiana* Schneider, 1875)[1].
Aggregata portunidarum Frenzel, 1885 (pars).
Benedenia octopiana (Schneider) Mingazzini, 1892, 1893.
Benedenia (Schneider) Labbé, 1895.
Klossia eberthi Labbé, 1896.
Klossia sepiana Labbé, 1896.
Klossia octopiana (Schneider) Siedlecki, 1898.
Benedenia octopiana (Schneider) Siedlecki, 1898.
Klossia eberthi (Labbé, 1896) } Labbé, 1899.
Aggregata portunidarum (Frenzel) }
Légeria Blanchard, 1900.
Legeria octopiana (Schneider) Doflein, 1901.
Eucoccidium octopianum (Schneider) Lühe, 1902.
Légerina eberthi (Labbé) Jacquemet, 1903.
Eucoccidium eberthi (Labbé) Lühe, 1903.
"*Benedenia*" *eberthi* (Labbé) } Minchin, 1903.
Aggregata portunidarum (Frenzel) }
Aggregata eberthi (Labbé) Léger & Duboscq, 1906.
Aggregata eberthi (Labbé) Moroff, 1908.
Aggregata arcuata [seu *arculata*] Moroff, 1908.
Aggregata mingazzinii [seu *mingazzini*] Moroff, 1908.
Aggregata minima Moroff, 1908.
Aggregata frenzeli Moroff, 1908.
Aggregata mamillana Moroff, 1908.

[1] The name *Benedenia octopiana* was originally given by Schneider (1875) to the parasites of *Octopus*. Later (Schneider, 1881) he referred these to the genus *Klossia*: and two years later still (Schneider, 1883) he included the parasites of *Sepia* in "*Klossia octopiana*" for the first time.

Aggregata (*Eucoccidium*) *eberthi* (Labbé) Léger & Duboscq, 1908.
Aggregata eberthi (Labbé) Doflein, 1911, 1916[1]; Dobell, 1914; Pixell-Goodrich, 1914; Dobell & Jameson, 1915; Joyet-Lavergne, 1924.

VI. THE NUCLEAR SYSTEM OF *A. EBERTHI*

In the foregoing descriptive section I have confined myself, as far as possible, to a bare record of observations: but as observations have to be interpreted in the process of making them, and words must be used to record them, it has been impossible to describe the development of *A. eberthi* in a wholly objective manner. I shall therefore discuss very briefly here one or two points which require qualification from this point of view.

From what has already been said it will be evident that the structure to which the name "nucleus" is given in *Aggregata* (and doubtless also in other Coccidia) is a much more complex organ than that to which the name is commonly applied (*e.g.* the nucleus of any somatic cell in a vertebrate). For this reason I prefer to speak of it as a "nuclear system" rather than as a "nucleus."

A whole individual *Aggregata*—whether male, female, or asexual—should be regarded, in my opinion, as a complete organism having a non-cellular structure. As an individual organism it is comparable with a whole metazoon—not with one of the cells in the body of a multicellular animal[2]. Consequently, I would describe *Aggregata* as a non-cellular organism possessing a nuclear system, and not as a cell containing a nucleus.

The nuclear system of *Aggregata* consists, as we have seen, of two chief parts—the large structure hitherto called "the nucleus," and within this a smaller structure which I have called "the micronucleus." This smaller structure develops in exactly the same manner in males, females, and schizonts. It arises as a minute granular body situated within the "nucleus" of the merozoite or sporozoite (respectively a young sexual or asexual organism), and grows into a small vesicular chromatin-containing structure having all the attributes of a typical "nucleus." The only objection to calling it a "nucleus" is that it is formed and develops within a structure to which this name is usually applied. It may therefore seem doubtful whether the "micronucleus" of *Aggregata* can properly be called a "nucleus" at all: but for want of a better term I shall continue to speak of it as such, while emphasizing the ambiguity involved in using such terminology.

In all adult parasites, whether sexual or asexual, the "nucleus" itself contains a large structure which—also for want of a better word—I shall continue to call "the karyosome." This structure, however, has a very complex composition[3], and is clearly not homologous with those bodies in the nuclei

[1] I may remark that the two original figures given by Doflein (1916), Fig. 851 *a* and *b*, p. 859, are scarcely recognizable as *A. eberthi*.

[2] See Dobell (1911).

[3] It will be apparent from what has already been said that I cannot wholly accept Siedlecki's (1905) views regarding either the structure or the functions of this organ in the Coccidia. If it be supposed, however, that his observations on *Caryotropha* (1905, 1907) are incomplete, it is not difficult to reconcile his general conceptions with the views which I have reached from studying *Aggregata*—a much larger and possibly more differentiated organism.

of Metazoa and many other Protozoa to which it is commonly applied. As we have seen, the micronucleus takes up its final position within the karyosome in sexual and asexual parasites alike. Every adult organism (see Text-fig. 2, p. 55) may therefore be described as binucleate, but with one of its nuclei lying within the karyosome of the other.

This description is admittedly equivocal: but so are the facts. I can describe them in no other way, unless I invent a new terminology. I hope that my descriptions and figures—especially the latter—will make my meaning clear, though some further elucidations are necessary.

That the "nucleus" of the merozoite or sporozoite of *Aggregata* is really a nucleus, everybody has hitherto admitted: consequently, it may be presumed that everybody will admit that the "nuclei" of the adult male, female, and schizont are also really nuclei—since they are merely older stages of the former. But the nuclei of the merozoite and the sporozoite are descendants of other nuclei, which, if we trace them back, are all derived from an original micronucleus. Consequently, this ancestral "nucleus," from which the descendant nuclei are ultimately derived, appears to have an equal right to the same name.

Consideration of the chromosomes will, perhaps, make this clearer. A chromatin-containing body which divides by mitosis, with a fixed number of individualized chromosomes, and all other essential attributes of a typical nucleus, will be generally allowed to be a real nucleus. Nobody will dispute the fact that the nuclei seen in the male during the early stages of gametogenesis, or in the sporont before spore-formation, are really such structures. But all of these are descendants of pre-existing micronuclei, from which their chromosomes were originally derived. The chromosomes of the male and female arise from, and are contained in, the micronuclei present in their karyosomes. These chromosomes unite in the zygote nucleus, and are afterwards distributed through all the nuclei of the sporont, the spores, and the sporozoites. The same set of chromosomes is present at all stages. The sporozoite's nucleus becomes that of a schizont: its chromosomes emerge from the micronucleus of the latter, and their descendants ultimately enter the nuclei of the merozoites. The micronucleus of the male, the female, and the schizont are therefore in the direct line of descent of other structures which are admittedly nuclei, and the lineage of the chromosomes passes at three points in the life-cycle directly through a micronucleus. Consequently, it seems reasonable to regard the micronucleus as a true nucleus, in the ordinary sense of the term.

If it be agreed that the micronucleus is as much a nucleus as any of the other chromosome-containing structures to which the name is applied, it will then become evident that another difficulty in interpretation arises when we come to consider the structure hitherto called a "nucleus" in the adult parasite (sexual or asexual). The "nucleus" of a male or female, for example, is derived by growth from the nucleus of a merozoite: and this nucleus contains a complete (haploid) set of chromosomes. But in the growth of the sexual

individual there is also differentiation, and of such a sort that the chromosomes —as is seen by their subsequent development—pass into the micronucleus. There is no evidence that the "nucleus" of an adult *Aggregata* contains, or is able to form, any chromosomes at all—apart from those in the micronucleus within it. Consequently, if chromosomes are a criterion of a nucleus, the micronucleus of *Aggregata* is a true nucleus, but the "nucleus" itself is not.

Nevertheless, the "nucleus" of the adult is directly derived from a pre-existing nucleus (that of a merozoite or sporozoite, as the case may be), and has many other morphological attributes of a true nucleus: while its "nuclear space" is the field wherein the chromosomes of the micronucleus undergo their manœuvres. It seems still reasonable, therefore, to regard the "nucleus" of *Aggregata* as the homologue of the nuclei of other organisms.

For these and other considerations I conceive it to be justifiable, after pointing out the limitations of the terms, to say that an adult *Aggregata* (male, female, or schizont) is really a binucleate organism.

Now the micronucleus of *Aggregata* contains the chromosomes and is the parent nucleus of all subsequent nuclei formed in the same individual (gamete nuclei or merozoite nuclei). It is therefore a "germinal" or "reproductive" nucleus comparable with the germ-cell nuclei of a metazoon. The nucleus itself, on the other hand, plays no part in the life of any individual (sexual or asexual) save that in which it is situated. In all cases it ultimately degenerates and dies with the individual to which it belongs. It is thus comparable with the somatic nuclei of a metazoon. Consequently, I think it is reasonable to say not only that *Aggregata* is a binucleate organism, but that one of its nuclei is a germinal nucleus and the other somatic—the former being contained within the latter.

It is well known that all typical Ciliophora possess two nuclei, or sets of nuclei, called meganuclei and micronuclei. In a given individual both have a common ancestry from one original nucleus. At conjugation the meganuclei perish, while the micronuclei form, by division, nuclei which are homologous with gamete-nuclei[1]: and the meganuclei have thus been recognized, since the time of Maupas, as equivalent to somatic nuclei, the micronuclei to germinal nuclei. The behaviour of the meganucleus and micronucleus in a ciliate is thus closely comparable with that of the nucleus and micronucleus of *Aggregata*. It therefore seems rational to homologize the nucleus of *Aggregata* with the meganucleus of a form such as *Paramecium*, and the micronucleus of *Aggregata* with the micronucleus of *Paramecium*. It is partly for this reason that I use the term micronucleus for the structure found in *Aggregata*: I believe it to be homologous with the structure of the same name observable in Ciliata. According to my view, an *Aggregata* is, in its nuclear constitution, strictly comparable with a ciliate; but with the difference that the micronucleus of

[1] A fuller exposition of the nuclear constitution of ciliates will be found in an earlier paper (Dobell, 1914*b*).

a ciliate is typically independent of the meganucleus, whereas in *Aggregata* it is contained within it.

We have, indeed, among the Ciliates themselves at least one organism which appears to have a similar nuclear system. *Ichthyophthirius*, according to the observations of Neresheimer (1908) and Buschkiel (1911), at certain stages possesses only a single nucleus. From this, subsequently, a smaller nucleus is budded off: and from the later development it is clear that the two nuclei so formed are respectively homologous with the meganucleus and micronucleus of other ciliates. At a still later stage the binucleate organism—after undergoing a series of nuclear changes interpreted as autogamy—becomes uninucleate once more, as a result of the micronucleus entering the meganucleus[1]. This apparently uninucleate organism is therefore, like other ciliates, really binucleate; but its micronucleus is contained within its meganucleus. I interpret the nuclear system of *Ichthyophthirius* at this stage as an exact counterpart of that of *Aggregata*.

Ichthyophthirius is exceptional among the Ciliates, and it is the only form—so far as I know—in which the micronucleus becomes inclosed within the meganucleus. In the vast majority of other known forms the two nuclei remain separate. In most of the Coccidia hitherto described, on the other hand, only a single nucleus has been distinguished—*Aggregata* forming at present an exception. But there is at least one coccidian which actually does possess two separate nuclei at most stages in its life-cycle. This is the remarkably interesting organism discovered by Ikeda (1914), and named by him *Dobellia binucleata*[2]. In this form, the male, female, and schizont possess two nuclei apiece ("principal nucleus" and "accessory nucleus" of Ikeda) which behave as a germinal nucleus and a somatic nucleus respectively. In gametogenesis and schizogony the germinal nucleus gives rise to the nuclei of the gametes or merozoites, as the case may be, and the somatic nucleus remains behind and perishes with the parental organism. In these respects the two nuclei of *Dobellia* appear to behave exactly like the two nuclei of *Aggregata*—the only difference being that in the former they are separate, whilst in the latter one is inclosed within the other. Consequently, I regard the nucleus of *Aggregata* as the homologue of the somatic ("accessory") nucleus of *Dobellia*, and the germinal ("principal") nucleus of *Dobellia* as homologous with the micronucleus of

[1] At Munich in 1908 I had an opportunity of examining some of Dr Neresheimer's preparations, which showed this singular phenomenon in a convincing manner.

[2] The observations made upon this organism have recently been called in question by Doflein (1916) and Reichenow (1920*a*), but in my opinion without much justification. My friend the late Prof. Ikeda made these observations in my laboratory, and I checked them personally at every stage. He was a very accurate observer and an excellent cytologist, and his descriptions were based upon a series of beautiful preparations. I am not prepared to say that his interpretation of every detail was absolutely correct, though in the main I believe it to have been so. In any case, there can be no doubt, in my opinion, that *Dobellia* is really a binucleate coccidian, and that its two nuclei behave as he describes them—one being somatic, the other germinal. The organism was very difficult to study at many stages on account of its extremely small size, but this particular point was demonstrable with certainty.

Aggregata: and moreover, as these structures appear to be comparable with the two kinds of nuclei seen in the Ciliata, I see no reason why the same terms should not be uniformly applied to all. I am therefore prepared to call both the "nucleus" of *Aggregata* and the "accessory nucleus" of *Dobellia* a meganucleus; and both the "micronucleus" of *Aggregata* and the "principal nucleus" of *Dobellia* a micronucleus—using these terms in the same sense as they are used when applied to *Paramecium*, and accepting all the implications involved in using such a terminology.

I am not yet prepared, however, to assert that all Coccidia are binucleate—like *Aggregata* and *Dobellia*—though I think it highly probable that the majority of these organisms, when more carefully investigated, will prove to have a nuclear system similar to that of *Aggregata*. In most Coccidia the micronucleus has probably been overlooked, I think, on account of its minute size and peculiar position: but further investigations are required to ascertain whether this is so or not, and the question may therefore be left open until more information has been obtained. The nuclear development in the very young stages has hitherto received but scant attention from those who have studied the Coccidia; and seeing that so striking a structure as the micronucleus of *Aggregata* has passed unobserved by many investigators, it would not be surprising if similar structures in other forms which are much harder to study have also been overlooked.

In his work on *Diplocystis* Jameson (1920) has shown that this gregarine also possesses a structure similar to the micronucleus of *Aggregata*, and he has discussed the previous observations made on similar structures, in other Gregarines, by others. I shall therefore say no more on this subject here, as I agree with his general conclusions. I will only note that, since the publication of his paper, additional—if somewhat meagre—evidence of the existence of a micronucleus in *Gregarina cuneata* has been furnished by Milojevic (1921).

It appears to me probable, therefore, that future research will show that in its nuclear system *Aggregata* is not peculiar among Coccidia, but that a similar nuclear organization exists not only in the many other Coccidia but even in the Gregarines also—in fact, in all the Sporozoa[1] properly so called (*i.e.* excluding the "Neosporidia"). All of these, indeed, may ultimately have to be regarded as binucleate and not uninucleate organisms. But at present, though there is much to justify such a view, generalization on this subject is not warranted.

The complex nuclear system of *Aggregata*—and of other Sporozoa—appears to me to supply a convincing demonstration of the absurdity of describing any such organism as "unicellular[2]." But I leave it to others, who believe in

[1] The peculiar binucleate "sporozoon" described under the name of *Drilosphaera* by Granata (1919) should perhaps be mentioned here. But in view of its doubtful life-cycle and questionable systematic position, I am at present unable to discuss its nuclear system in relation to that of *Aggregata*. It seems to me not unlikely that it will ultimately prove to be more nearly related to the Actinomyxidia than to the Sporozoa (Coccidia and Gregarines).

[2] Cf. Dobell (1911). The above remark is also equally applicable to the nuclear systems of the Ciliophora.

the cell theory and the primitiveness and simplicity of the Protozoa, to reconcile the structural organization of *Aggregata* with their tenets, and to frame a definition of "a cell" which will be equally applicable to an *Aggregata* and to the morphological units composing the body of an adult metazoon.

VII. FERTILIZATION AND REDUCTION IN *A. EBERTHI* AND OTHER COCCIDIA

In earlier pages I have given a full description of the process of fertilization and the meiotic phenomena in *A. eberthi*. It remains for me to say something about the observations of other workers on the corresponding stages in other Coccidia.

Up to the time when I published my preliminary note[1], in 1915, nothing was definitely known about the phenomena of reduction in the Coccidia. Earlier workers, such as Siedlecki (1899) for *Adelea* and Schaudinn (1900) for *Eimeria*, observed "reduction" in various species during the maturation of the macrogamete: but it was obvious that the process so called was not a true reduction (halving of the chromosome number) but merely an elimination of "chromatin" from the nucleus. It now seems probable, indeed, that it was simply a misinterpretation of certain stages in the fragmentation and degeneration of the karyosome. Schaudinn (1902) also described two "reduction divisions" of the macrogamete nucleus before fertilization in *Cyclospora*: but here also no chromosomes were counted, or even observed; and the correct interpretation of this observation still remains obscure. I need not discuss the "reduction" described in "*Adelea*" by Jollos (1909), since his observations have already been demolished by Reichenow and Schellack (1912). The last two authors have themselves published a series of important papers on the Coccidia, but up to 1915, when their account of *Adelea ovata* appeared, they had been unable to ascertain how reduction occurs in any of the forms which they studied.

At this time there was a general impression that reduction must occur in the Coccidia during gametogenesis—an impression based on analogy with the Metazoa, though no actual observations or chromosome counts could be cited in support of it. Indeed, the work of Schellack and Reichenow supplied some direct evidence[2] which made it appear probable that no reduction occurs during gametogenesis: but they were unable to ascertain when or how reduction is effected, and the suggestions which they put forward were wide of the mark. Subsequently Reichenow (1920, 1921) published an important paper on the Haemogregarines of lizards, and in this he recorded a number of observations on *Karyolysus*—several species—in which he had found a chromosome cycle

[1] Dobell and Jameson (1915).

[2] See especially Schellack and Reichenow (1915). This paper, which appeared shortly before my preliminary note, did not come to my notice until long afterwards; and I gather that the German workers remained equally unaware of my work until some years later. This was due in both cases, of course, to the state of war prevailing at that time.

identical with that which I had previously recorded in *Aggregata*. In *Karyolysus* the chromosomes appear to be four or five (probably the latter) in number, and to be present in this number at every nuclear division except that immediately following fertilization, at which there are eight or ten. Reichenow states that he had come to this conclusion before he was aware of my results in the case of *Aggregata*, and his work therefore affords strong confirmation of my findings.

When we turn to other species, the evidence is less satisfactory. In certain forms, such as *Orcheobius herpobdellae* (Kunze, 1907), *Klossia vitrina* (Moroff, 1911), *Merocystis kathae* (Foulon, 1919), and *Adelea ovata* (studied by several workers), the chromosomes have been seen at various stages, and sometimes counted more or less definitely. Many of the stages described and figured in these organisms bear a close resemblance to certain stages in *Aggregata*, and I cannot doubt that further work on all of these organisms will ultimately show that they develop in a closely similar manner. I say this with some confidence with regard to *Adelea ovata* and *Orcheobius*, as they are forms which I have myself studied[1].

The case of *Adelea ovata* is specially instructive. Siedlecki (1899) and the earlier workers—including myself (1907)—saw little of the chromosomes, though they were acquainted with the life-cycle in its general outline. But Debaisieux (1911) observed some additional details, and Schellack and Reichenow (1915) were able to record still more. They were not able, however, to give any definite information regarding the chromosome cycle, though they concluded—no doubt correctly—that no reduction occurs during gametogenesis in either the male or the female. Still more recently Greiner (1921)—who, curiously enough, appears to have been unaware of the work of Schellack and Reichenow (1915)—has made an important contribution to our knowledge of this species. She finds that the chromosome number is constant (four or five) during schizogony, and that the same number is present during both nuclear divisions in microgametogenesis[2] and occurs again later in the sporogonic divisions. It is clear, therefore, that this organism is haploid, and that reduction does not occur during gametogenesis but after fertilization—as in *Aggregata*. The details of the meiotic phases have not been satisfactorily

[1] The chromosome numbers are, however, probably different. The (haploid) number in *Adelea ovata* appears to be five. In *Orcheobius*, Kunze (1907) did not succeed in counting the chromosomes, but to judge from certain of his figures (and my own preparations) their number is seven or eight.

[2] In my opinion the formation of the microgametes in *A. ovata* has never yet been accurately described. Siedlecki's account is incorrect, and that which I gave in 1907 is incomplete; while Schellack and Reichenow evidently did not even find many of the stages which I described. The description given by Greiner is, however, almost complete. From a re-examination of my old preparations, in the light of my observations on *Aggregata*, I am now certain that the microgametogenesis of *Adelea* is closely similar to that of *Aggregata*. Instead of two ordinary mitotic divisions taking place, a tetraster figure is formed—like the polyaster in *Aggregata*—which gives rise to four nuclei. Greiner's figures accord perfectly with this view, though they are not so interpreted.

worked out, however, and Greiner's observations on this part of the life-history are difficult to interpret[1]; but as far as they go, they appear to justify the belief that the chromosome cycle of *Adelea*—though not the number of chromosomes—is identical with that of *Aggregata*.

So far as I know, nobody has yet studied any coccidian in as much detail or for so long as I have studied *Aggregata*. The stages which other workers have found are mostly those which occur fairly commonly in preparations, and they correspond with those which I found in *Aggregata* during the first few years of my work. Most of the forms investigated are also much smaller and less favourable for study than *Aggregata*: and consequently I think it likely that my results with this organism will be found to agree fairly closely with those ultimately obtained in the study of other Coccidia.

I do not assert, however, that the details of development in all Coccidia are probably identical with those which I have found in *Aggregata*: for if Reichenow's observations on *Karyolysus* (1920, 1921) are correct, there is already good evidence that the details of development differ in different species. The essential similarity lies in the chromosome cycle as a whole: and as the Coccidia form a very homogeneous group, it is probable that all of them are haploid organisms; that all undergo reduction immediately after fertilization; and that all[2] possess, therefore, a chromosome cycle like that of *A. eberthi*.

Too little is known about the details of nuclear development in most Coccidia for discussion of them to be profitable at the present moment. But a fairly full account of *Karyolysus* has been given by Reichenow, and I shall therefore make a few remarks on his observations here.

Reichenow (1920, 1921) claims[3] that his work on *Karyolysus* has shown that there is a close agreement between the various stages of meiosis in the Coccidia and those seen during maturation of the germ-cells in Metazoa and Metaphyta. He says this agreement is observable even down to details, and believes that his observations "explain the theoretical significance" of the "fertilization spindle." It must be noted that the process of fertilization and reduction described by Reichenow in *Karyolysus* differs in many respects from my account of the same phenomena in *Aggregata*. There is, however, general agreement in the following points: (1) after the fusion of the gametes, the zygote nucleus forms a "fertilization spindle"; (2) this then disappears; and (3) the chromosomes, which have united in homologous pairs, undergo

[1] She found 4–5 chromosomes in the gamete nuclei, and 8–10 in the early zygote nucleus: but afterwards she counted 16–20, though only 4–5 were present in later stages of sporogony and in schizonts. The tetraploid stages require elucidation.

[2] I include in this generalization the so-called Haemosporidia. These organisms are so obviously similar to the Coccidia that it appears to me almost certain that their chromosome cycles are identical. The "reduction" described in the malaria parasites before fertilization is obviously a misinterpretation like that made in the case of many coccidia. *Plasmodium* is probably a haploid organism like *Aggregata*, and undergoes no reduction before fertilization.

[3] Cf. Reichenow (1921), p. 214.

disjunction at the ensuing mitosis. The zygote nucleus is diploid, but the daughter-nuclei derived from it are all haploid once more. Several different species of *Karyolysus*—which differ in details—are simultaneously described, but for none of them is a complete series of figures of all the stages given.

I must confess that the details of meiosis in *Karyolysus*, as described by Reichenow, do not seem to me to show a striking similarity to the meiotic phases of any multicellular animal or plant with which I am acquainted. The end result is the same—a halving of the chromosome number: but the detailed mechanism of this process appears to me very different. The difference is even more striking, I think, in the case of *Aggregata*; and I cannot help thinking that this is partly because Reichenow has not seen every stage in *Karyolysus*. In *Aggregata*, as we have seen, the chromatin of the two gametes becomes inextricably commingled in the zygote nucleus shortly after fertilization. From this mixed chromatin the peculiar "fertilization spindle" figure is formed, consisting of an elongate net-like arrangement of threads having no regular pattern. The number of the threads varies, and there is no justification for calling them "chromosomes." The "spindle" figure gives place to that which I have called the "cobweb" figure, in which there is no trace of chromosomes—the chromatin being finely divided once more. From the "cobweb" a spireme is formed, consisting of a single thread—not a double (diplotaene) thread. The thread contracts, then opens out into a series of loops, which break apart at diakinesis as 12 filamentar chromosomes consisting of six homologous pairs. They actually pair—*a* with *a*, *b* with *b*, etc.—as they take up their position on the equatorial plate; and the disjunction of the pairs into univalent elements occurs at the ensuing metaphase. We may agree to regard the stage when the spireme is in the form of a single slender thread as a leptotaene stage, and we may call the contraction figure which follows "synapsis": but where are the diplotaene and pachytaene and other stages seen in the Metazoa and Metaphyta? How are we to homologize all the various events which occur during two successive divisions in these organisms with those which occur during only one in *Aggregata*?

Reichenow has not answered these questions, but he does make one specific comparison. He says that he has discovered the "significance" of the "fertilization spindle": and his "explanation" of this peculiar figure—characteristic of Coccidia alone, as far as is known—is that it represents the stage at which parallel conjugation of the chromosomes occurs. Unfortunately, I cannot find any evidence of a parallel conjugation of chromosomes in any figures of "fertilization spindles" which he has yet published—either in *Karyolysus*, *Adelea*, or any other form. My own preparations of *Aggregata*[1] and the figures of *Adelea* published by Greiner (1921) appear to me to show clearly that no parallel conjugation occurs at this stage. I am therefore unable to adopt Reichenow's view. Moreover, even if it should ultimately be proved that his interpretation is correct—for *Karyolysus* or any other forms—

[1] And also of *Adelea*, *Orcheobius*, and other coccidia.

it is difficult to see how it affords any "explanation" of the "fertilization spindle." If the "spindle" is a formation resulting from or accompanying the stage of parallel conjugation of the chromosomes, how is it that no such figure is seen at the corresponding stages in other organisms?

In my view the meiotic phases in the Coccidia—and also in the Gregarines—are peculiar, and are not at present exactly comparable, step by step, with those seen in the Metazoa and Metaphyta. When it is remembered that the meiotic phenomena in these organisms have been studied for years by an army of workers, and that no general agreement has yet been reached regarding many matters of detail, it is obviously rash to make detailed comparisons with the Coccidia, about which only a few facts—the work of a small squad of workers—are definitely known. All that can be said with certainty at present is that reduction does occur in the Coccidia; but that it occurs at a different stage in the life-history from that at which it takes place in the Metazoa, and is probably effected in a different manner. In the Coccidia, there is only one reduction division—not two—and it occurs immediately after—not before—fertilization; these peculiarities being associated with the circumstance that the Sporozoa are haploid organisms—not diploid, like the Metazoa.

VIII. SOME GENERAL CONSIDERATIONS ARISING FROM THE STUDY OF THE CHROMOSOMES OF *A. EBERTHI*

If we compare the chromosome cycle of *A. eberthi* with those of other organisms, a number of interesting points at once suggest themselves. I shall not attempt to discuss all these here, as I hope to deal with them elsewhere: but a few general remarks may not be out of place at this point.

It is clear that *Aggregata* is a haploid organism at every stage in its life-history except one, which is probably of brief duration. Every male, female, and asexual parasite (schizont) contains a single set of six unpaired chromosomes, whereof each member is individually differentiated. The same haploid set of chromosomes is found in every microgamete and every macrogamete nucleus, and no chromosome reduction (halving) occurs during the formation of the gametes of either sort. When the gametes fuse, the product is therefore diploid: its nucleus (a zygote nucleus) contains 12 chromosomes, consisting of six homologous pairs. But at the first division of this diploid nucleus—the first nuclear division of the sporont—disjunction of the pairs takes place, so that the daughter-nuclei and their descendants become haploid once more. Reduction, or halving of the chromosomes, thus occurs immediately after fertilization—not during gametogenesis, as it does in the Metazoa.

We have in *Aggregata* an organism which shows conspicuous polymorphism combined with chromosomic identity of its various forms: we have an animal with a regular and well-marked alternation of generations—sexual and asexual—and strongly developed sexual dimorphism; yet all individuals—whether male, female, or asexual—and all gametes—whether formed by males (microgametes) or from females (macrogametes)—are apparently identical in

chromosomic constitution. Every individual or gamete contains the same haploid set of six differentiated chromosomes in its nucleus[1]. Every nuclear division of *A. eberthi*, throughout the whole life-cycle, is mitotic: and every chromosome—so far as it has been possible to study them—retains its individuality throughout. The largest (*a*) chromosome, for example, can be found with comparative ease at every nuclear division. It is certainly present, unpaired, in the nucleus of every organism—male, female, or schizont—and in that of every gamete (microgamete or macrogamete) and every sporozoite and merozoite. The same can be said with certainty of the *b* chromosome—also fairly easily recognizable at all stages: and it is probably true of all the others (*c–f*), though the smallest members of the group (*e* and *f*) cannot always be demonstrated with absolute certainty at every stage, on account of their minute size. They are present at certain critical stages, however, and there is no reason to suppose that they disappear at intermediate stages.

It is thus clear that in its chromosomic constitution *Aggregata* presents several novel and interesting features[2]. We have here an organism in which the "somatic" number of chromosomes is identical in both sexes. There is nothing, as regards chromosome form or number, to distinguish the male from the female—as happens so often in the Metazoa: and there is nothing in the chromosome make-up of the individual to distinguish the alternative sexual and asexual generations from one another—as in so many plants. Further, neither males, females, nor asexual parasites are zygotes. Asexuality and sexual dimorphism are here seen in haploid organisms, with a chromosomic constitution like that of the gametes of a metazoon: while the gametes themselves possess the same haploid set of chromosomes as the organisms which produce them. The zygote is a stage of very brief duration, lasting for the period of one nuclear division only—the first division of the sporont, which for the major part of its life is, like the sexual individuals and the schizont, a haploid organism. If we exclude this brief event in the life-cycle then we may say, in summary, that: (1) the merozoites (which are young sexual parasites, produced asexually by fission of a schizont); (2) the male and female individuals into which they grow; (3) the highly differentiated microgametes

[1] When I say that the chromosomes are "the same" or "identical" I use these terms, of course, in the loose way in which they are usually applied in this connexion. When six individually differentiated chromosomes enter into the formation of a resting nucleus at the telophases of division, and six similar chromosomes appear in the same nucleus during the prophases of the next and then divide into two apparently identical sets, each of which enters a daughter nucleus, it is convenient to speak of all these sets of six as "identical." But I do not mean to imply that the telophase chromosomes are really the very same as those formed in the ensuing prophases, or that either group of daughter chromosomes is, in a strict sense, the same as the parent group from which it is formed by division. The first supposition would be, in most cases, unwarranted, and the second manifestly absurd.

[2] Wasielewski (1924) has recently remarked that the reduction phenomena seen in *Aggregata* differ from those characteristic of the Metazoa only in "the time of their occurrence"—being otherwise "völlig vergleichbar." This seems to me entirely to miss the point. The chromosome cycle of *Aggregata* is not the same as that of a metazoon, with trifling variations in detail, but radically different.

and macrogametes formed by these; (4) the sporont, resulting from the fusion of a pair of gametes; (5) the spores, produced by fission of the sporont; (6) the sporozoites formed within the spores; (7) the schizonts, which are asexual individuals formed by growth from sporozoites—all these stages in the life-history, notwithstanding their morphological and physiological diversity, are haploid forms with an identical chromosomic constitution.

For those who believe, from a study of the Metazoa and Metaphyta, that the chromosomes—any or all of them—"determine" morphological characters, "bear" hereditary properties, or constitute "the physical basis of heredity," the foregoing facts should afford ample matter for reflexion.

There is now, moreover, considerable evidence to show that, as regards the general features of its chromosome cycle, *Aggregata* does not stand alone among organisms. Since the publication of our preliminary note on the subject[1], the observations have been confirmed in the case of other coccidia (*Karyolysus*) by Reichenow (1920, 1921); and I think there can be little doubt, from the recent observations of Greiner (1921), that the chromosome cycle of *Adelea* also is essentially similar to that of *Aggregata*. There is thus evidence that in both Eimeriidea and Adeleidea the same remarkable chromosome cycle obtains.

Furthermore, Jameson (1920)[2] has shown conclusively that a similar chromosome cycle occurs in at least one member of the Gregarinida—*Diplocystis schneideri*; and he has also shown that earlier evidence to the contrary—based upon incomplete observations on other Gregarines and false analogies with the Metazoa—is mostly unsound. As I fully agree with his conclusions in this respect, I will only add that the recent publications of Tolosani (1916) and Bastin (1919) upon *Monocystis* do not contain anything[3] which leads me to believe that the views previously expressed by Jameson and myself in this connexion are erroneous. Despite the doubts of Reichenow and other critics, I still maintain that "The chromosome cycle is, as a rule, so constant a character in any natural group of animals or plants, that we find every reason to believe at present that the chromosomes of the Sporozoa generally will be found—when more fully studied—to behave like those of *Aggregata* and *Diplocystis*" (Dobell and Jameson, 1915).

Concerning the chromosome cycle in other Protozoa we know, with certainty, but little at the present moment. It can be said confidently, however, that the foregoing general statement is not applicable to the Ciliata. From the observations which have been made[4] by Prandtl upon *Didinium*, by

[1] Dobell and Jameson (1915).

[2] Originally announced by Dobell and Jameson (1915).

[3] Similar criticisms to those which I and Jameson (1915) and Jameson (1920) applied to the work of Mulsow (1911)—on another species of *Monocystis*—are applicable to these two papers, which were both written, apparently, in ignorance of our results. There is at present no conclusive evidence that *Monocystis* is radically different from *Diplocystis*, as regards its chromosome cycle: but the possibility that this may be so cannot be ignored.

[4] For references to some of the more important papers on this subject see Dobell (1914*b*).

Popoff upon *Carchesium*, and by others upon a variety of other ciliates, it is now fairly certain that reduction takes place in these organisms at a stage corresponding to that of gametogenesis in a metazoon. The Ciliata are diploid organisms, and as regards their chromosome cycle they resemble the Metazoa—not the Sporozoa. Of the Rhizopoda we know little in this respect: but the recent observations of Bělař (1923) show that at least one of the Heliozoa—*Actinophrys*—is diploid, and that its chromosome cycle is like that of a ciliate or a metazoon. The Mycetozoa are probably also diploid, if Jahn's (1911) interpretation of the plasmodium is correct. Little is known about the chromosomes of the Mastigophora; but some of the Phytoflagellata (*e.g. Chlamydomonas*[1], and perhaps *Volvox*) are probably haploid, like the Sporozoa, and halve their chromosomes immediately after fertilization.

Among organisms other than Protozoa the haploid condition probably also predominates in certain forms. Allen (1905) showed—more or less conclusively—that reduction occurs in *Coleochaete* immediately after fertilization, and similar observations have since been made on *Spirogyra* and some other Algae; while the more recent work of Claussen (1912) upon *Pyronema* and of Burgeff (1915) on *Phycomyces* has revealed comparable conditions in these organisms. From these and other observations[2] it can hardly be doubted, therefore, that some Algae and Fungi possess a chromosome cycle similar to that of the Sporozoa.

It is thus clear that the remarkable chromosome cycle found in *Aggregata* is not an isolated exception which can be dismissed as an anomaly. On the contrary, it is now evident that there are many similar haploid organisms—so many, indeed, that they cannot be ignored by those who desire to generalize concerning the function of the chromosomes. Nearly all current conceptions about chromosomes are based upon the behaviour of these bodies in the Metazoa and Metaphyta; and consequently they apply to these organisms only. It is therefore not too much to say that, when more is known about the chromosome cycles of the Protista, many a generalization which now seems plausible and acceptable will have to be abandoned as a too hasty conclusion drawn from insufficient data.

I shall say no more on this subject here. I will only beseech those who now confidently generalize about chromosomes and their function—especially in relation to the phenomena of heredity—to consider the case of *Aggregata* and similar haploid organisms before they build further upon their present precarious foundations.

[1] Cf. Pascher (1916).

[2] A useful list of references to the chief works relating to Algae will be found in Bonnet (1914). The botanical text-books speak glibly of x and $2x$ generations in numerous Algae and Fungi; but anybody who will take the trouble to study the original sources of their statements will find that they are seldom supported by adequate evidence based upon accurate chromosome counts at all stages in the life-cycle.

IX. SUMMARY OF THE LIFE-HISTORY AND CHROMOSOME CYCLE

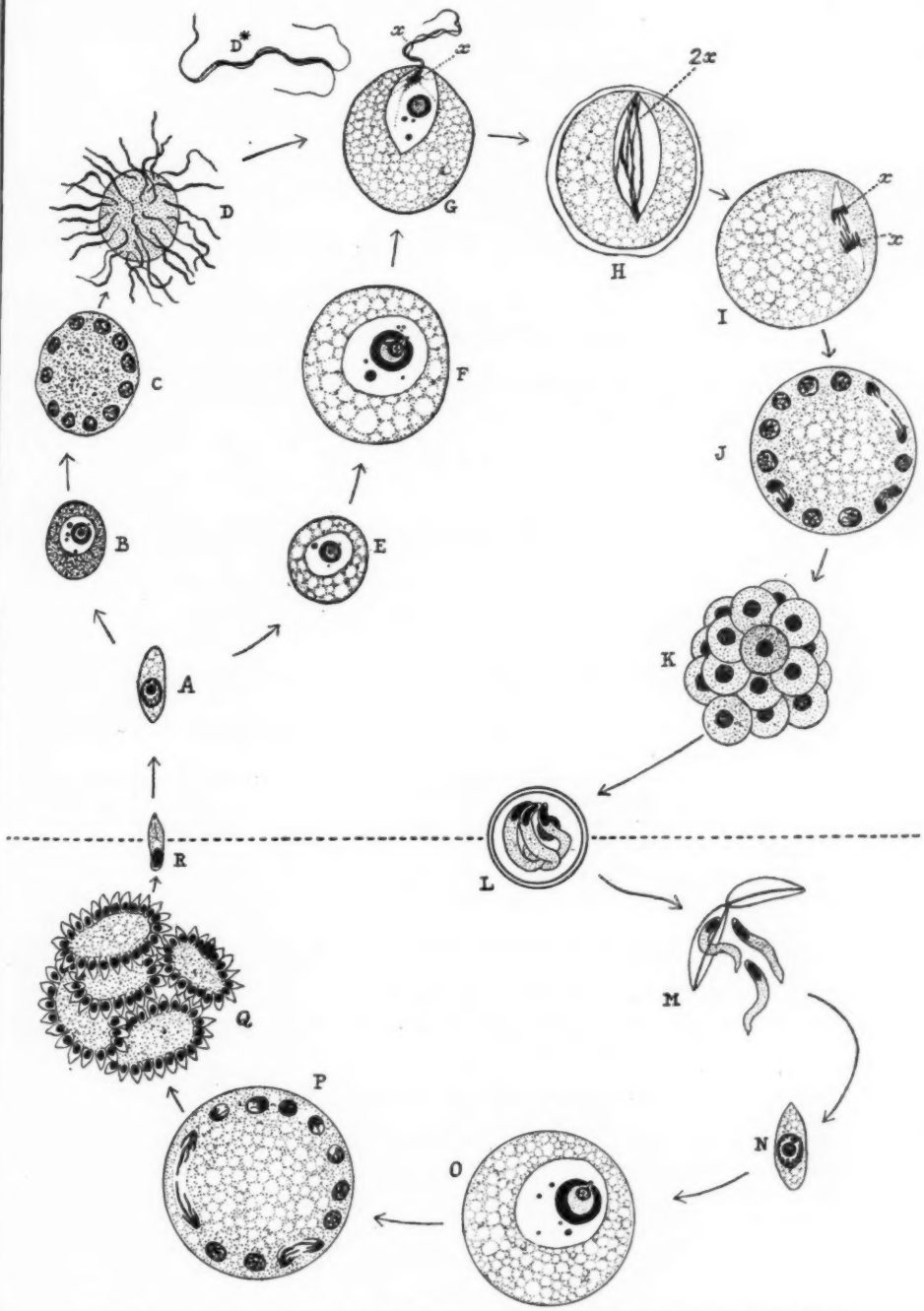
The life-history of *Aggregata eberthi* is summarized in the accompanying diagram (Text-fig. 3). The stages shown above the dotted line occur in *Sepia officinalis*, those below in *Portunus*.

Merozoites (*R*), when swallowed by the cuttlefish, pass through the epithelium and enter the submucous tissue of the caecum or intestine, where they develop into young (undifferentiated) parasites (*A*) which grow into males (*B*) or females (*E*). The former, after undergoing repeated nuclear divisions (*C*), produce broods of microgametes (*D*)—each of which (*D**) has a complicated structure. Females (*E*), on the other hand, are bodily converted into macrogametes (*F*), which undergo a complex series of nuclear changes during maturation. No reduction occurs during gametogenesis.

Fertilization (*G*) is effected by the union of one microgamete with one macrogamete, and is followed by the formation of a fertilization membrane which, in part, gives rise to the oocyst. The zygote (*H*) becomes a sporont, which, after repeated division of its nucleus (*I*, *J*), ultimately segments—leaving no residuum—into a large number of spherical uninucleate sporoblasts (*K*). Each of these is then converted into a spore (*L*) containing three sporozoites and a small sporocystic residue.

The ripe spores pass out of the intestine with the cuttlefish's excrement; and when swallowed by a *Portunus* they open in its intestine and liberate their contained sporozoites (*M*). These penetrate the epithelium of the crab's midgut, and enter the subepithelial connective tissue, where they grow into schizonts (*N*, *O*). Finally, the schizonts undergo schizogony, producing large broods of merozoites (*P*, *Q*). No further development takes place unless the schizogonic cysts, in the crab's coelome, are eaten by a cuttlefish: in which case they liberate their contained merozoites (*R*) and the development just described then begins anew.

A. eberthi is, at every stage but one in the whole life-cycle, a haploid organism. Every nuclear division is mitotic: and every nucleus (except the zygote nucleus) contains a single set of six differentiated and unpaired chromosomes. In the zygote nucleus alone there are 12 chromosomes—six homologous pairs—derived from the two gamete nuclei: but at the first division of this nucleus (first division in sporont, Text-fig. 3, *I*) the chromosomes are again halved to six. Reduction (meiosis) thus occurs immediately after fertilization. No halving of the chromosome number occurs during gametogenesis or schizogony; and males, females, and asexual parasites all possess an identical chromosomic constitution. Every nucleus in Text-fig. 3 is therefore an x nucleus, with the exception of stage *H*, which is $2x$. (Between stages *G* and *I* many nuclear changes occur, but only one—the so-called "fertilization spindle"—is figured (*H*) in the diagram.)



Text-fig. 3. Diagram representing the life-cycle of *Aggregata eberthi*. (Stages above the dotted line occur in *Sepia*, stages below in *Portunus*.)

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DESCRIPTION OF PLATES I—VI.

All drawings have been made with the aid of the *camera lucida*, and the magnifications recorded are exact. (The optical apparatus employed is noted on p. 17.) All figures are of *Aggregata eberthi*. (Figs. 1–166 show stages in *Sepia officinalis*: Figs. 167–199 stages in *Portunus depurator*.) The following abbreviations are used throughout to denote the methods by which the specimens illustrated were fixed or stained, as the case may be:

B.C.	=Grenacher's borax carmine.
Bo.	=Bouin's picro-formol-acetic fixative.
Del.Hx.	=Delafield's haematoxylin.
$FeCl_3Hn.$	=Alcoholic ferric-chloride haematein stain (modification of Mallory's method).
FeHn.	=Alcoholic iron-alum haematein (C.D.).
Fl.	=Flemming's fixative (strong).
H.	=Mayer's haemalum.
Heid.FeHx.	=Heidenhain's aqueous iron-alum haematoxylin.
Lg.	=Light green.
MB.Eo.	=Mann's methylblue-eosin stain (modified).
Or.G.	=Orange G.
P.A.	=Picro-acetic acid fixative.
ParaC.	=Mayer's paracarmine.
S.A.	=Schaudinn's sublimate-alcohol fixative.
Saf.	=Safranin.
S.F.	=Sublimate-formol fixative.
Sect.	=Section (thickness indicated in microns).
Sm.	=Smear preparation (*i.e.* drawing made from a whole parasite).

PLATE I.

Fig. 1. Three cells from the caecal epithelium of *Sepia officinalis*, with a very young parasite lying in the middle one. (Sm.) Fl. Saf. ×1000.

Figs. 2, 3, 4, 5, 6, 7. Successive stages in the growth and differentiation of young parasites in caecal submucous tissue of *Sepia*. (Sm.) Fl. Saf. ×1000.

Fig. 8. Very young parasite from same site. (Sm.) Fl. Saf. ×2000.

Fig. 9. Very young parasite lying within its host-cell in submucous tissue. (Sm.) Fl. Saf. ×1000.

Fig. 10. Young parasite in host-cell—a young columnar epithelium cell of mucous membrane. (Sect. 5μ.) S.A. Del.Hx. Or.G. ×2000.

Fig. 11. Young (older) parasite lying in a host-cell in the subepithelial connective tissue of gut wall. (Sect. 5μ.) S.A. Del.Hx. Or.G. ×336.

Fig. 12. Nucleus only of young parasite, showing micronucleus applied to micropyle of karyosome. (Sm.) Fl. Saf. ×2000.

Fig. 13. Nucleus only of a larger (female) parasite, showing micronucleus passing through micropyle. (Sm.) Fl. Saf. Lg. ×2000.

Fig. 14. Whole young female parasite, with micronucleus now inside karyosome. (Sm.) Fl. Saf. Lg. ×1000.

Fig. 15. Nucleus only, young female parasite. Micronucleus inside karyosome, which is forming(?) "buds." (Sm.) Fl. Saf. ×1000.

Figs. 16, 17. Two consecutive thin sections (3μ) of nucleus only of a small immature female with only one large karyosome. 16 shows micropyles of karyosome; 17 shows micronucleus inside karyosome. S.A. Saf. Lg. ×1000.

Fig. 18. Nucleus only, largish immature female. (Sect. 5μ, cut somewhat tangentially through karyosome.) Showing micronucleus in micropyle. S.A. Del.Hx. Or.G. ×1000.

Fig. 19. Nucleus only, largish immature female; showing micronucleus inside main karyosome, and several daughter karyosomes. (Sect. 5μ.) S.A. Del.Hx. Or.G. ×1000.

Figs. 20, 21, 22. Nuclei only, maturing females; showing stages in disintegration of micronucleus and formation of nuclear network. (Sect. 5μ.) S.A.—Fig. 20, Saf. Or.G.—Fig. 21, Saf. Lg.—Fig. 22, Del.Hx. Or.G. All ×1000.

Fig. 23. Very thin tangential section of karyosome of large female (becoming a macrogamete), showing disintegrating micronucleus embedded in its wall. S.A. Saf. Lg. ×2000.

Fig. 24. Nucleus only, maturing macrogamete. Whole nucleus of small parasite. (Sect 18μ.) Showing nuclear network (early stage) and eight karyosomes. S.F. Del.Hx. ×2000.

Fig. 25. Whole (immature) macrogamete, small specimen; showing later stage of nuclear network, multiple karyosomes, etc. (Sect. 20μ.) S.F. FeHn. ×1000.

Fig. 26. Nucleus only, macrogamete at stage similar to Fig. 25. Entire nuclear network drawn as accurately as possible. (Sect. 18μ, whole nucleus.) S.F. ParaC. ×2000. (Details of karyosome structure not drawn.)

Fig. 27. Karyosome only; large macrogamete at stage slightly earlier than that shown in Fig. 26. (Sect. 18μ.) S.F. ParaC. ×1000. (Optical section of whole main karyosome.)

Fig. 28. Nucleus only, smallish macrogamete. Spireme stage—the thread drawn as accurately as possible, but its central tangle almost undrawable, and its loops not countable with certainty. (Sect. 18μ, containing whole nucleus.) S.F. Del.Hx. ×2000.

Fig 29. End (containing nucleus) of macrogamete at slightly later stage. Spireme loops forming. (Sect. 18μ.) S.F. FeHn. Lg. ×1000.

Fig. 30. Nucleus only, small macrogamete. Formation of the chromosomes from looped threads of spireme. (Sect. 20μ, containing whole nucleus.) S.F. FeHn. ×1000.

Fig. 31. Nucleus only, small macrogamete. The six chromosomes fully formed. (Sect. 18μ, containing whole nucleus.) S.F. ParaC. ×2000.

Fig. 32. Nucleus only; later stage, with the six chromosomes lying in long axis of the elongated nucleus and forming the "pre-fertilization spindle." Whole nucleus reconstructed from three consecutive sections (5μ)—the six chromosomes all lying in one section and only the karyosomes in those adjacent. S.A. Saf. ×2000.

Fig. 33. Nucleus of macrogamete and surrounding cytoplasm. Chromosomes passing to pole of elongated nucleus (seen obliquely) and beginning to break up. (Sect. 18μ.) S.F. FeHn. ×1000.

Fig. 34. Whole macrogamete, almost mature. Nucleus elongated and polar. Chromosomes broken down, and their chromatin granules taking up their position at the peripheral pole of the nucleus. Karyosome fragmented. (Some of the karyosome fragments added from adjacent section, in order to show entire nuclear contents.) (Sect. 20μ.) S.F. FeHn. ×1000.

PLATE II.

Figs. 35, 36. Small immature female parasite and rather large immature male, respectively, lying side by side in same preparation: both at approximately same stage of development. (Sect. 20μ.) S.F. FeHn. ×1000.

Fig. 37. Very young male parasite, with micronucleus in micropyle of karyosome. (Sect. 20μ.) S.F. FeHn. ×2000.

Fig. 38. Young male parasite (large), showing micronucleus in hollow karyosome, etc. (Sect. 5μ.) S.A. Saf. Lg. ×1000.

Fig. 39. Nucleus only, small male. Formation of nuclear net. (Sect. 20μ.) S.F. FeHn. ×2000.

Fig. 40. Nucleus only, very small male. Spireme stage. (Sect. 20μ.) S.F. FeHn. ×2000.

Fig. 41. Nucleus only, male; spireme breaking into six loops (chromosomes). (Sect. 20μ.) S.F. H. ×2000.

Fig. 42. Nucleus only, smallish male. The six filamentar chromosomes fully formed. (Sect. 5μ.) S.A. Saf. Lg. ×2000. (This nucleus actually contains another large karyosome, which is present in the adjacent section.)

Fig. 43. Part of small male parasite, with nucleus at a later stage. Chromosomes passing to achromatic body. (Sect. 20μ.) S.F. H. ×2000.

Fig. 44. Later stage; chromosomes reaching achromatic body. (Sect. 20μ.) S.F. H. ×2000.

Fig. 45. Chromosomes only, first spindle, male (large). (Sect. 18μ, spindle cut across obliquely.) The six chromosomes are well separated and are becoming short and thick. S.F. FeHn. ×2000.

Fig. 46. Periphery of male, later stage. Equatorial plate of 1st spindle cut across, showing the six chromosomes—now shorter and thicker. Spindle not shown. (Sect. 20μ.) S.F. H. ×2000.

Fig. 47. Periphery of male, showing the six globular chromosomes on equatorial plate of 1st spindle. Spindle cut obliquely and only one end seen. (Sect. 20μ.) S.F. H. $\times$2000.

Fig. 48. First spindle, male; six globular chromosomes dividing into 12—metaphase. (Sect. 20μ.) S.F. H. $\times$2000.

Fig. 49. Whole male parasite; showing early anaphase, 1st spindle. (The spindle lies superficially in reality—the organism being viewed from the pole at which it lies.) Parasite surrounded by its collapsed capsule. (Sect. 20μ.) S.F. H. $\times$2000.

Fig. 50. Periphery of male, slightly later anaphase. Chromosomes becoming drawn out. (Sect. 20μ.) S.F. H. $\times$2000.

Fig. 51. Small male, at later stage—late anaphase of 1st spindle (beginning of polyaster). Chromosomes splitting. (Whole parasite in one thick section, 20μ.) S.F. FeHn. $\times$2000.

Fig. 52. Surface view of nuclear elements only, at similar stage. Large male. (Sect. 20μ.) S.F. FeHn. $\times$2000.

Figs. 53, 54. Portions of periphery of male parasites at successive later stages. Formation of polyaster. (Sect. 20μ.) S.F. FeHn. $\times$2000.

Fig. 55. Part of nucleus and one end of male parasite, early stage of polyaster. Showing asters, and splitting of the chromosome threads (part only drawn—three more asters and chromosome groups actually present). (Sect. 20μ.) S.F. H. $\times$2000.

Fig. 56. Very small male. Polyaster stage, 1st division. (Sect. 20μ.) S.F. FeHn. $\times$2000. (The surrounding line represents the outline of the capsule, which is in reality much thicker. The tangled chromosomes forming the centre of the polyaster are drawn as accurately as possible; but their real disposition is even more complex than it appears, for more asters are present —above and below—than it is possible to show in this figure.)

Fig. 57. Surface view of later stage—chromosome groups (eight) emerging from polyaster. Very small male. (Sect. 20μ.) S.F. FeHn. $\times$2000. (Parasite not quite entire—a small portion of it being contained in adjacent section.)

Fig. 58. Larger male, later stage; formation of ten nuclei from polyaster. Some nuclei are more advanced than others, but in the earlier ones the six chromosomes are clearly countable. (Sect. 20μ.) S.F. FeHn. $\times$2000. (Entire organism not shown—part being contained in an adjoining section.)

Fig. 59. Part of periphery of large male, showing two nuclei (resting) formed after break-up of polyaster figure. (Sect. 5μ.) S.A. Saf. Lg. $\times$2000. (The nuclei are stained a very pale red, while the tiny projecting "centrosomes" are in reality green.)

Fig. 60. Small male, second (bipolar) division period. One nucleus (N.E.) is resting, two others (S.E. and S.) show spireme formation, and the other three (N.W., N., and S.W. respectively) show successive stages in division. (Sect. 10μ.) S.F. FeHn. $\times$2000. (Part only of parasite contained in this section.)

Fig. 61. Anaphase group of chromosomes, 2nd nuclear division in very large male. (Sect. 8μ.) S.A. $FeCl_3Hn$. $\times$2000.

Fig. 62. Small portion of periphery of a male parasite, showing five nuclei: later stage than Fig. 60. (Sect. 20μ.) S.F. FeHn. $\times$2000.

Figs. 63, 64, 65. Three successive stages of nuclear division in male parasites during later stages. Nuclei only. From sections: 63 and 64 (20μ), S.F. FeHn.; 65 (8μ), S.A. $FeCl_3Hn$. $\times$2000.

Fig. 66. Last nuclear division (before gamete-formation) in male. Anaphase groups. (Sect. 8μ.) S.A. $FeCl_3Hn$. $\times$2000.

Fig. 67. Small cyst of ripe microgametes (from caecum). Whole. (Sm.) S.A. Del.Hx. $\times$320. One residual body present.

Fig. 68. Large cyst of ripe microgametes (from intestine). Whole. (Sm.) S.A. H. $\times$320. Five residual bodies present.

PLATE III.

Figs. 69, 70, 71, 72, 73, 74, 75. Successive stages in the formation of microgametes: small portions of periphery of male parasites. (Sect.) 69, 70, 72=S.F. FeHn.: 71, 73, 74, 75=S.A. $FeCl_3Hn$. $\times$2000.

Figs. 76, 77. Mature microgametes—free in lymph-spaces in connective tissue. 76a=trans. sec. in nuclear region, 76b=trans. sec. in post-nuclear region. (Sect.) S.F. FeHn. $\times$2000.

Fig. 78. Fertilization: entry of microgamete into nucleus of macrogamete. Cellular investment of latter shown. Fertilization membrane forming. (Sect. 20μ, some karyosomes added from next sect.) Viewed somewhat obliquely from pole of fertilization. S.F. FeHn. $\times$1000.

Fig. 79. Fertilization: later stage, microgamete in funnel. Very small parasite in caecum. (Sect. 18μ.) S.F. Del.Hx. $\times$1000.

Fig. 80. Fertilized macrogamete (part) shortly after entry of microgamete. Fertilization membrane formed: cellular investment partly shown. (Sect. 20μ.) S.F. FeHn. $\times$1000.

Figs. 81, 82. Partial drawings of two macrogamete nuclei (fertilization pole only), shortly after fertilization: showing changes undergone by nucleus of microgamete. (Sect. 20μ.) S.F. FeHn. $\times$2000.

Fig. 83. Nucleus only, fertilized macrogamete at later stage; seen obliquely from fertilization pole. Chromatin of the two gametes almost completely fused into a stellate mass of granules. (Sect. 20μ.) S.F. FeHn. $\times$2000.

Figs. 84, 85. Successive later stages—nuclear end of zygote only. Formation of "fertilization spindle." (Sect. 20μ.) S.F. FeHn. $\times$1000.

Fig. 86. Main karyosome (degenerating) of large macrogamete shortly after fertilization. (Sect. 5μ.) S.A. Saf. Or.G. $\times$1000.

Fig. 87. Formation of "fertilization spindle." Whole nuclear contents (small zygote) shown. (Sect. 20μ.) S.F. FeHn. $\times$1000.

Figs. 88, 89. Small and large zygotes respectively, showing fully formed "fertilization spindles." (Sect. 20μ.) S.F. FeHn. $\times$1000.

PLATE IV.

Fig. 90. "Cobweb figure"—following "fertilization spindle." Whole nucleus shown; small zygote. (Sect. 20μ.) S.F. FeHn. $\times$1000.

Fig. 91. Later stage: optical section of nucleus at level of achromatic spindle-rudiment. (Sect. 20μ.) S.F. FeHn. $\times$1000.

Fig. 92. Later stage: contraction of net round achromatic body. Whole nucleus shown. (Sect. 20μ.) S.F. FeHn. $\times$1000.

Fig. 93. Slightly later stage. Optical section of nucleus at level of achromatic body. Oocyst (formed by fusion of fertilization membrane and cellular investment) partly shown. (Sect. 20μ.) S.F. FeHn. $\times$1000.

Fig. 94. Nucleus only, complete. Spireme opening out into 12 loops. (Sect. 20μ.) S.F. FeHn. $\times$2000.

Fig. 95. Zygote nucleus only, later: showing spireme segmented into 12 filamentar chromosomes (six unequal pairs of homologous chromosomes). Karyosome fragments not all drawn. (Sect. 20μ.) S.F. FeHn. $\times$2000.

Fig. 96. Later stage, showing chromosomes massed and passing towards achromatic body. (Sect. 20μ.) S.F. FeHn. $\times$1000.

Fig. 97. Similar stage, showing details of achromatic spindle. Very large zygote. Chromosomes not all visible. (Sect. 16μ.) S.F. FeHn. $\times$1000.

Fig. 98. The 12 filamentar chromosomes passing on to equatorial plate—first nuclear division of sporont. Small parasite. (Sect. 20μ.) S.F. FeHn. $\times$2000.

Fig. 99. Achromatic spindle and chromosomes (not all shown): first division of sporont nucleus. (Sect. 20μ.) S.F. FeHn. $\times$2000.

Fig. 100. Chromosomes passing on to equatorial plate of fully-formed spindle: first division of sporont. (Sect. 16μ.) S.F. FeHn. $\times$1000.

Fig. 101. Oblique section of first spindle, later stage. Chromosomes becoming massive. (Sect. 20μ.) S.F. FeHn. $\times$2000.

Fig. 102. First spindle of sporont nucleus. Fully formed and complete, seen from side. (Sect. 7·5μ.) S.A. Saf. Lg. $\times$2000.

Fig. 103. The six bivalent chromosomes on equatorial plate of Fig. 102, drawn separately. $\times$2000.

Fig. 104. First spindle, chromosomes massed together and massive. (Sect. 20μ.) S.F. FeHn. $\times$2000.

Fig. 105. First spindle, chromosomes vesicular. (Sect. 8μ.) S.A. $FeCl_3Hn$. $\times$2000.

Fig. 106. The vesicular chromosomes of Fig. 105, drawn separately. $\times$2000.

Fig. 107. Metaphase, first division. Disjunction of the six bivalent chromosomes into two homologous groups of six univalents. Chromosomes only; spindle lying somewhat obliquely. (Sect. 20μ.) S.A. H. $\times$2000.

Fig. 108. Early anaphase, first division. Small parasite. (Sect. 20μ.) S.F. FeHn. $\times$1000.

Fig. 109. The six anaphase chromosomes at a stage similar to that of Fig. 108, drawn separately. (Sect. 20μ.) S.F. FeHn. $\times$2000.

Figs. 110, 111. Early anaphase chromosome groups, first spindle: polar views. (Sect. 5μ and 20μ.) Small caecal parasites. S.A. Saf. Lg. and S.F. FeHn. respectively. $\times$2000.

Fig. 112. Early anaphase group, and pole of spindle: side view; large parasite. (Sect. 20μ.) S.F. FeHn. $\times$2000.

Figs. 113, 114, 115. Later anaphases, successive stages. 113 and 114, small parasites: 115, large. (Sect. 20μ.) S.F. FeHn. $\times$1000.

Fig. 116. Triaster (early stage of polyaster), very small sporont. (Sect. 20μ.) S.F. FeHn. $\times$1000.

Fig. 117. Tetraster figure (early polyaster). Small sporont. (2 Sect. 20μ, combined. Whole parasite and oocyst shown.) S.F. FeHn. $\times$1000.

Figs. 118, 119. Chromosome groups (six in each) emerging from first division figure (polyaster), small sporonts. (Sect. 20μ: each figure reconstructed from two sections.) S.F. FeHn. $\times$1000.

Fig. 120. Nuclei (four) entering first resting stage—after polyaster. Small sporont. (2 sect. 20μ, combined.) S.F. FeHn. $\times$1000. (One nucleus—which partly overlaps another—has been slightly displaced, in order to show its structure more clearly.)

PLATE V.

Fig. 121. Large sporont, late stage in first division (polymitosis). Whole organism reconstructed from three sections. (Sect. 16μ.) S.F. FeHn. $\times$1000.

Fig. 122. Chromosome group and aster from a large sporont—end stage of polymitosis. (Sect. 16μ.) S.F. FeHn. $\times$2000.

Fig. 123. Chromosome group from large sporont during polymitosis stage. The aster has divided and the chromosomes are splitting. (Sect. 16μ.) S.F. FeHn. $\times$2000.

Fig. 124. Later stage: separation of daughter chromosomes and asters. The six chromosomes are recognizable in either anaphase group. (Sect. 16μ.) S.F. FeHn. $\times$2000.

Fig. 125. A single chromosome group and achromatic body at end of polyaster stage—about to form resting nucleus. (Sect. 20μ.) S.F. FeHn. $\times$2000.

Fig. 126. The chromosomes from a similar group (small sporont), drawn separately. (Sect. 20μ.) S.F. FeHn. $\times$2000.

Figs. 127, 128, 129. Successive telophase stages; formation of resting nucleus—after polymitosis —in large sporonts. (Sect. 20μ.) S.F. FeHn. $\times$2000.

Fig. 130. Three resting nuclei (after polymitosis) at periphery of large sporont. (Sect. 12μ.) S.F. FeHn. $\times$1000.

Fig. 131. Small sporont with six resting nuclei (after polymitosis). (2 sect. 20μ, combined to show whole organism.) S.F. FeHn. $\times$1000.

Fig. 132. Small sporont. Nuclei dividing—early (bipolar) divisions after first resting stage (following polymitosis). (2 sect. 20μ, combined.) S.F. FeHn. $\times$1000.

Fig. 133. Medium-sized sporont, at later stage, showing superficial folding, etc. Whole organism. (Sm.) S.A. Del.Hx. $\times$800.

Fig. 134. Large sporont, later stage. (Sect. 6μ.) P.A. B.C. $\times$360.

Figs. 135, 136. Successive early stages in mitosis of sporont nuclei—second division period. (Sect. 20μ.) S.A. FeHn. $\times$2000.

Fig. 137. Anaphase chromosome group, late division in sporont. (Sect. 8μ.) S.A. $FeCl_3Hn$. $\times$2000.

Fig. 138. Small portion of sporont, segmenting into sporoblasts. (Sect. 7·5μ.) S.A. Saf. Lg. $\times$2000.

Fig. 139. Mammiform sporoblasts, fully formed. (Sect. 20μ.) S.A. FeHn. $\times$2000.

Fig. 140. Sporoblasts becoming spores: showing first spore coat (epispore), reserve bodies, etc. (Sect. 8μ.) S.A. $FeCl_3Hn$. $\times$2000.

PLATE VI.

Figs. 141–166. Development of spores. (Spore coats not drawn in many figures—the sporoplasm only being shown.)

Fig. 141. Uninucleate spore; showing epispore and endospore etc. (Sect. 7·5μ.) S.A. Saf. Lg. ×2000.

Fig. 142. Prophase, first sporal division. (Sect. 5μ.) S.A. Saf. Lg. ×2000.

Fig. 143. Spireme, first sporal division. (Sect. 5μ.) S.A. Saf. Lg. ×2000.

Fig. 144. Diakinesis, first sporal division. The six chromosomes filamentar, and clearly recognizable. (Sect. 7·5μ.) S.A. Saf. Lg. ×2000.

Fig. 145. Chromosomes on equatorial plate, first sporal division. (Sect. 5μ.) S.A. Saf. Lg. ×2000.

Fig. 146. Globular chromosomes on equatorial plate: first sporal division. (Sect. 5μ.) S.A. Saf. Lg. ×2000.

Fig. 147. First sporal division, spindle with equatorial plate in side view. (Sect. 5μ.) S.A. Saf. Lg. ×2000.

Fig. 148. First sporal division: equatorial plate, metaphase. (Sect. 5μ.) S.A. ParaC. Lg. ×2000.

Fig. 149. First sporal division: early anaphase. (Sect. 5μ.) S.A. Saf. Lg. ×2000.

Fig. 150. Later anaphase chromosome group, first sporal division. (Sect. 7·5μ.) S.A. Saf. Lg. ×2000.

Fig. 151. Late anaphase, first sporal division. (Sect. 7·5μ.) S.A. Saf. Lg. ×2000.

Fig. 152. Early telophases, first sporal division. (Sect. 7·5μ.) S.A. Saf. Lg. ×2000.

Fig. 153. Sporoplasm with two resting nuclei; completion of first sporal division. (Sect. 7·5μ.) S.A. Saf. Lg. ×2000.

Fig. 154. Spireme; prophase of second sporal division. (Sect. 5μ.) S.A. Saf. Lg. ×2000.

Fig. 155. Spindle with equatorial plate, second sporal division. (Sect. 20μ.) S.A. MB.Eo. ×2000.

Fig. 156. Chromosomes only, equatorial plate of second spindle. (Sect. 7·5μ.) S.A. Saf. Lg. ×2000.

Fig. 157. Metaphase chromosomes, equatorial plate of second sporal division. (Sect. 5μ.) S.A. ParaC. Lg. ×2000.

Fig. 158. Late anaphases, second sporal division. (Sect. 5μ.) S.A. Saf. Lg. ×2000.

Fig. 159. Sporoplasm with three resting nuclei; completion of second sporal division. (Sect. 7·5μ.) S.A. Saf. Lg. ×2000

Fig. 160. Segmentation of sporoplasm into three sporozoites and residual body. (Sect. 20μ.) S.F. H. ×2000.

Fig. 161. Later stage. Differentiation of sporozoites. (Sect. 20μ.) S.A. MB.Eo. ×2000.

Fig. 162. Spore similar to preceding, viewed from "animal pole." (Sect. 20μ.) S.A. MB.Eo. ×2000.

Fig. 163. Fully formed spore, with three sporozoites and small residual body. (Sect. 20μ.) S.F. H. ×2000.

Fig. 164. Mature spore, viewed from nuclear pole. (Sect. 20μ.) S.F. H. ×2000.

Fig. 165. Tetrazoic spore (abnormal). (Sect. 20μ.) S.A. MB.Eo. ×2000.

Fig. 166. Hexazoic spore (abnormal). (Sect. 20μ.) S.A. MB.Eo. ×2000.

Fig. 167. Spore opening and liberating its three sporozoites in gut of *Portunus depurator*. (Sm.) S.A. ParaC. ×2000.

Fig. 168. Sporozoite in epithelial cell of midgut of *P. depurator*. (Sect. 14μ.) P.A. ParaC. Lg. ×2000.

Fig. 169. Small portion of section of midgut of *P. depurator*; showing sporozoites arrested by the unusually thick basal membrane. (Sect. 10μ.) S.A. B.C. ×800.

Fig. 170. Sporozoite penetrating basal membrane. (Sect. 10μ.) S.A. FeHn. Lg. ×2000.

Fig. 171. Sporozoite which has just passed through basal membrane into submucous tissue. (Sect. 10μ.) S.A. FeHn. Lg. ×2000.

Fig. 172. Sporozoite free in gut of *P. depurator*. (Sm.) S.A. ParaC. ×2000.

Fig. 173. Sporozoite (young schizont) in subepithelial tissue of midgut of *P. depurator*: third day after hatching from spore. (Sect. 14μ.) P.A. ParaC. Lg. ×2000.

Fig. 174. Young schizont. (Sect. 14μ.) P.A. ParaC. Lg. ×2000.

Fig. 175. Young schizont lying in a cell in the submucous tissue of the midgut of *P. depurator*. (Sect. 14μ.) P.A. ParaC. Lg. ×2000.

Fig. 176. Young schizont, showing micronucleus entering karyosome. (Sect. 5μ.) S.A. ParaC. ×2000.

Fig. 177. Nucleus only, small schizont (older than the preceding): showing micronucleus within the karyosome. (Sect. 5μ.) S.A. ParaC. ×2000.

Fig. 178. Nucleus only, medium-sized schizont: showing structure of karyosome, and micronucleus lying within it. (Sect. 10μ.) Bo. ParaC. Lg. ×1000.

Fig. 179. Large schizont. (Sect. 14μ.) S.A. FeHn. ×320.

Fig. 180. First spindle, early anaphase: schizont. (Sect. 14μ.) S.A. FeHn. ×1000.

Fig. 181. Chromosome group, end of first division (polymitosis) in schizont. (Sect. 14μ.) S.A. FeHn. ×2000.

Fig. 182. Chromosomes entering resting nucleus—end of first division (polymitosis) in schizont. (2 sect. 14μ, combined.) S.A. FeHn. ×2000.

Figs. 183, 184. Chromosome groups (late anaphases) from early nuclear divisions in schizonts. (Sect. 14μ.) S.A. Heid.FeHx. ×2000.

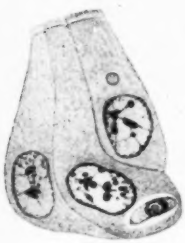
Figs. 185, 186, 187, 188. Anaphase chromosome groups from later divisions in schizonts. (Sect. 14μ.) S.A. (185, 186) Heid.FeHx. and (187, 188) ParaC. ×2000.

Figs. 189, 190, 191, 192, 193. Anaphase groups from later divisions in schizonts. (Sect. 14μ.) S.A. FeHn. ×2000.

Figs. 194, 195, 196, 197. Small portions of schizonts undergoing schizogony: showing four successive stages in the formation of merozoites. (Sect. 14μ (194, 195, 196) and 5μ (197).) S.A. Heid.FeHx. (194, 195, 196) and Bo. ParaC. Lg. (197). ×2000.

Fig. 198. Fully formed merozoites, lying freely in cyst—detached from residual protoplasm. (Sect. 10μ.) S.A. FeHn. ×2000.

Fig. 199. Complete transverse section of midgut of *P. depurator*, with four cysts containing merozoites. (Sect. 6μ.) S.A. B.C. ×60.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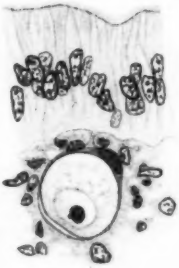
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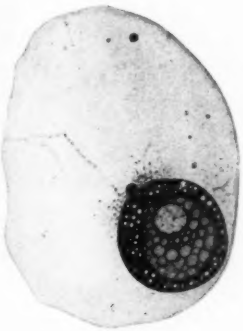
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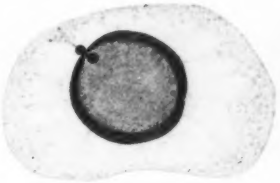
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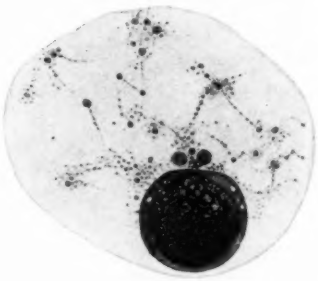
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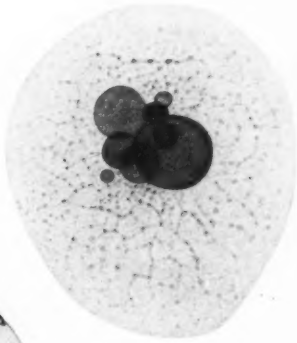
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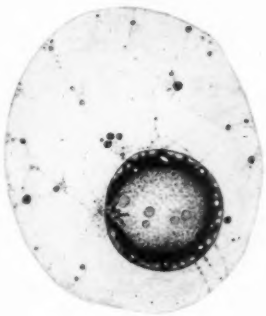
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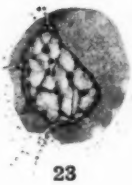
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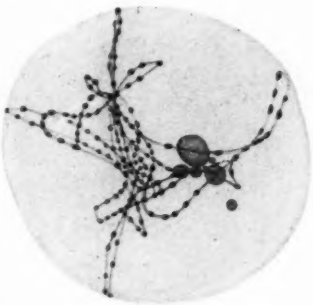
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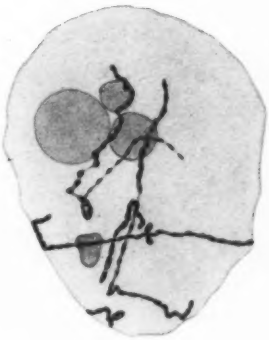
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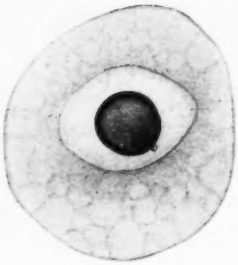

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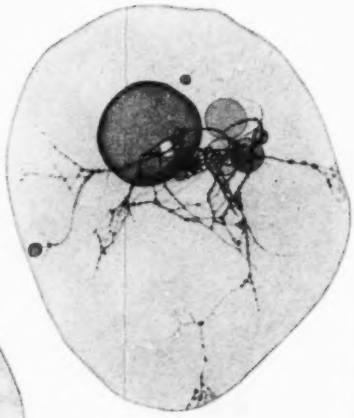

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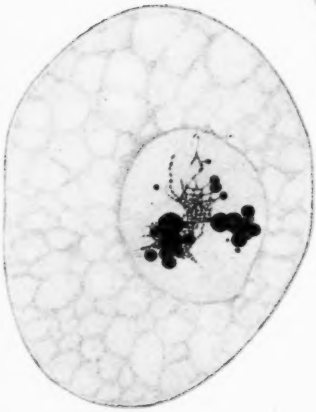

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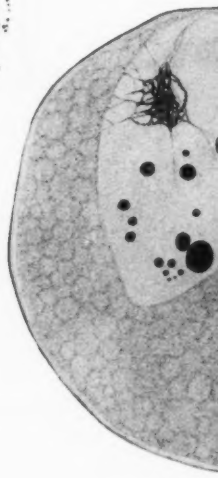

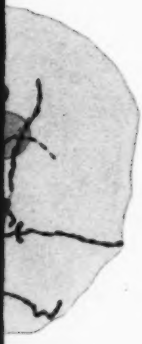

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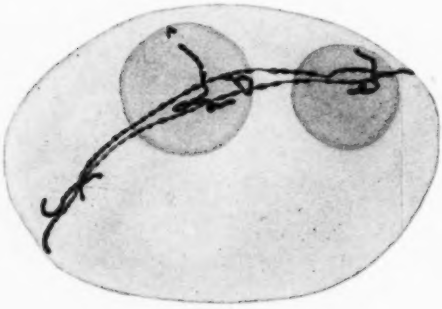

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PLATE I

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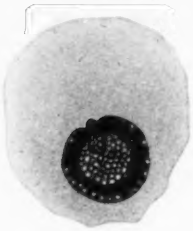

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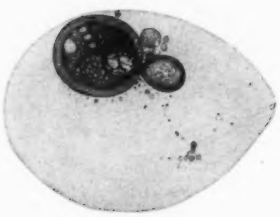

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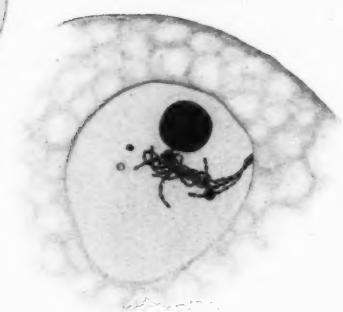

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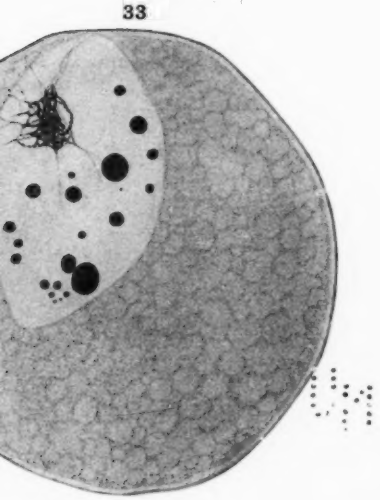

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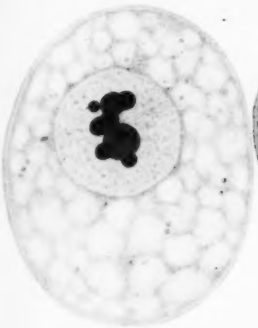

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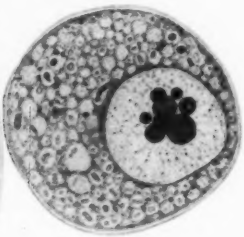

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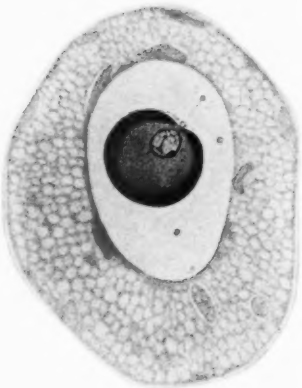

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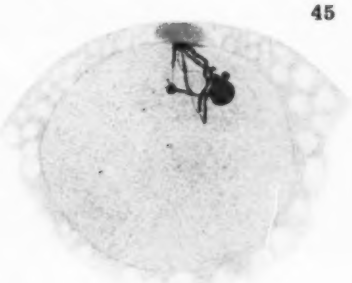

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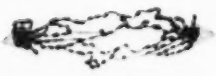

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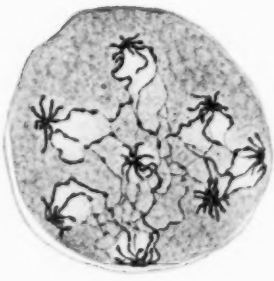

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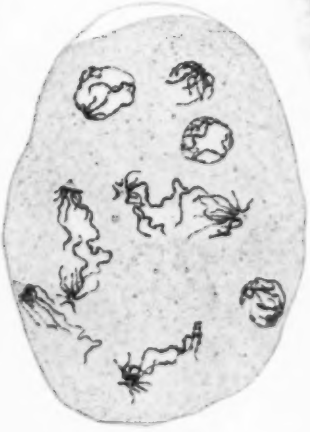

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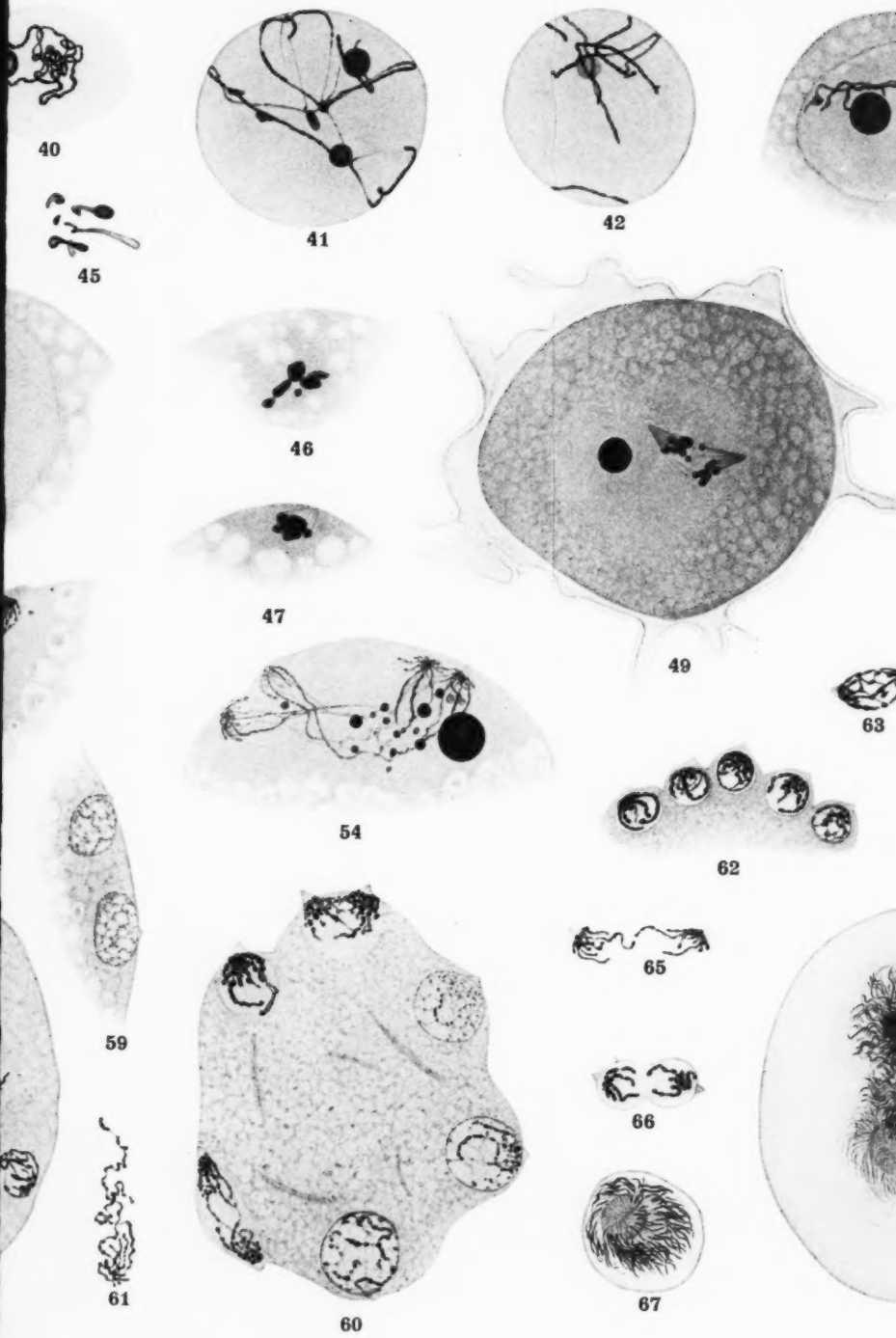
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PLATE II

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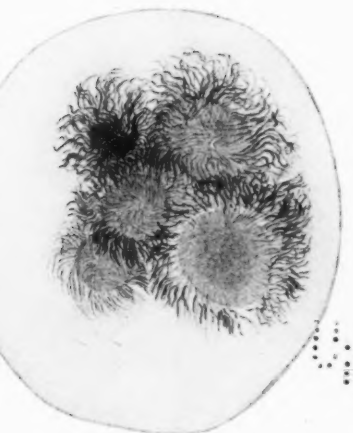

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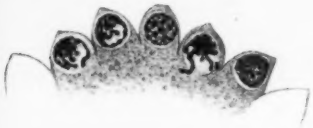

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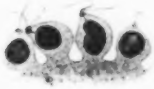

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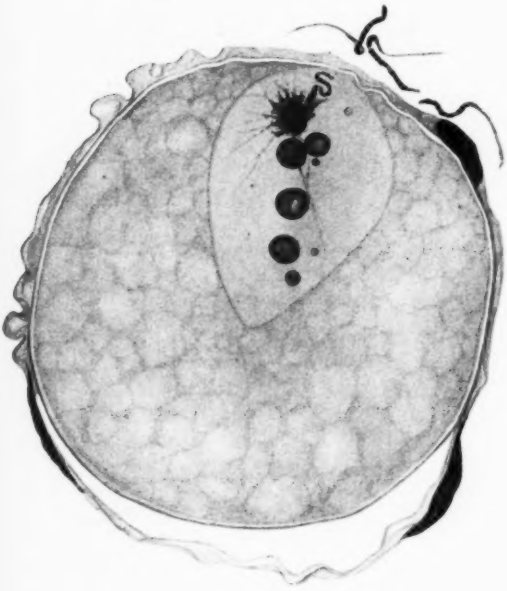

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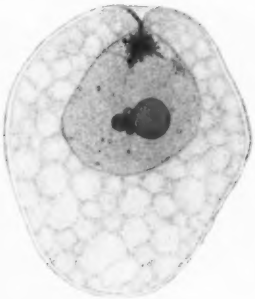

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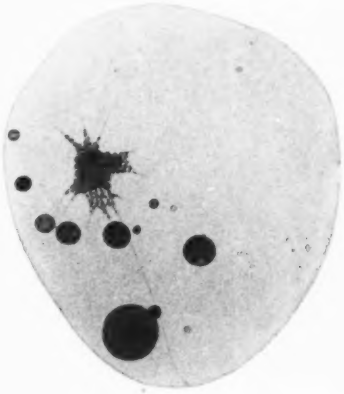

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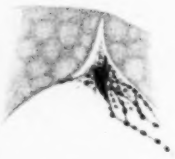

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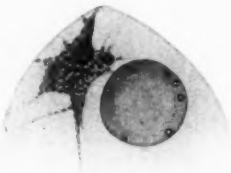

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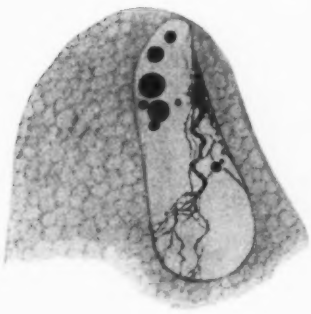

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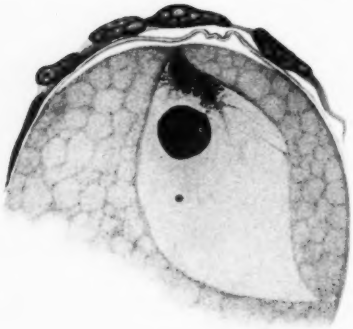

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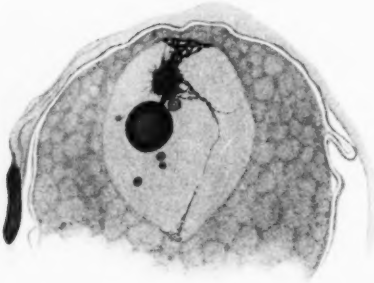

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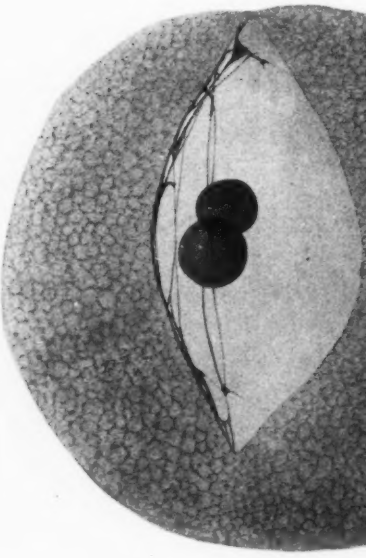

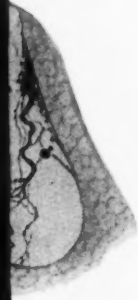

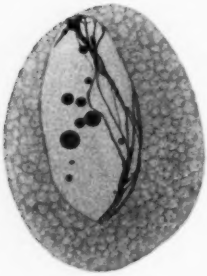

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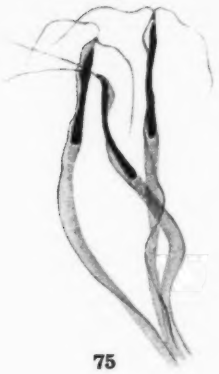

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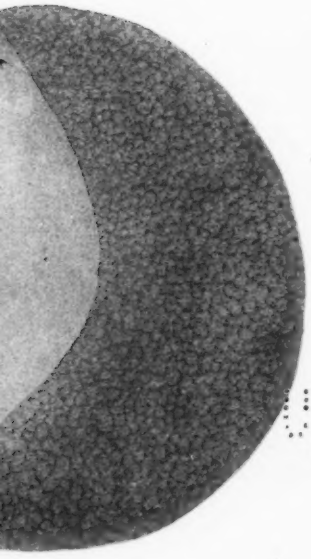

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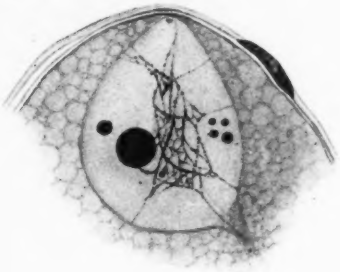

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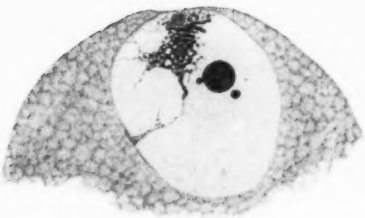

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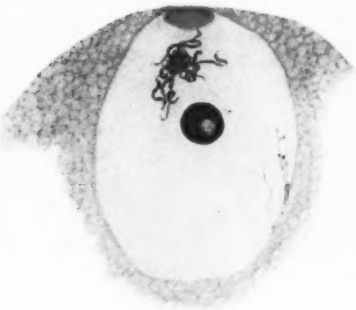

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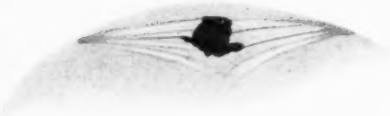

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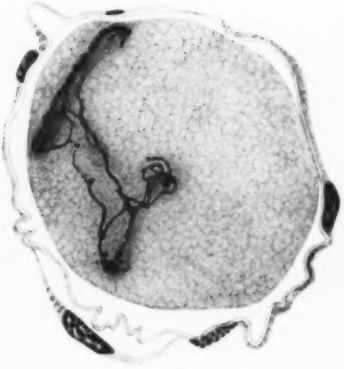

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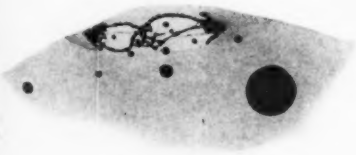

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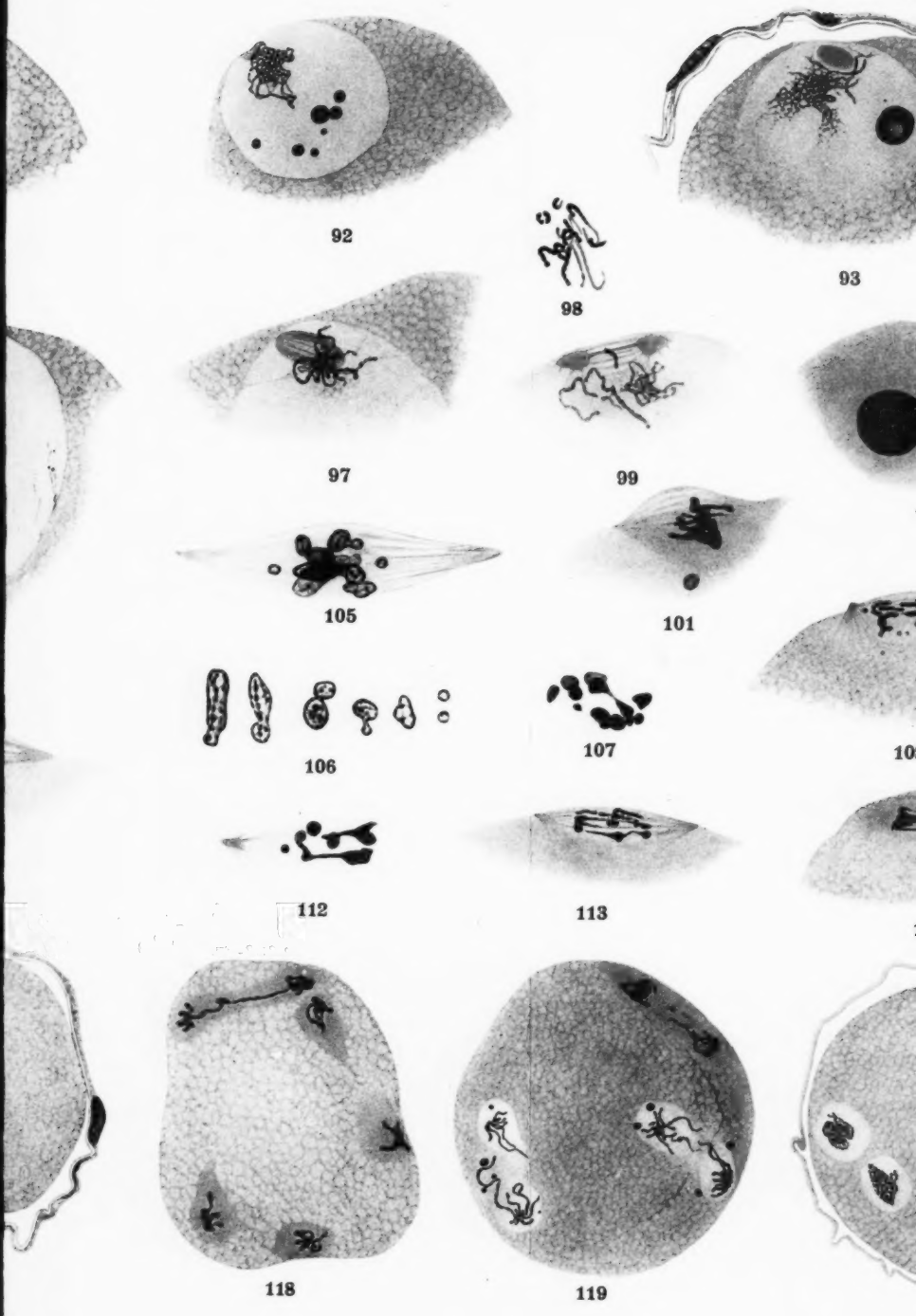
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PLATE IV

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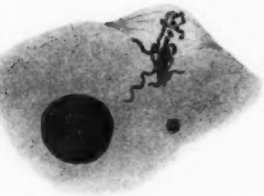

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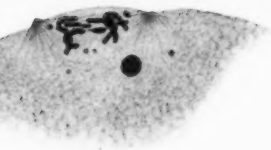

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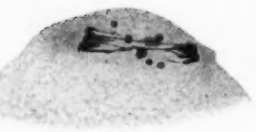

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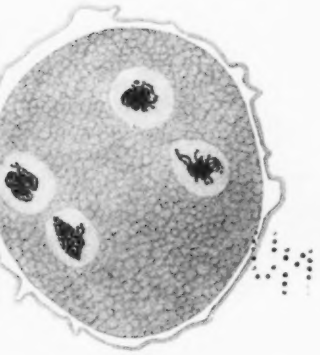

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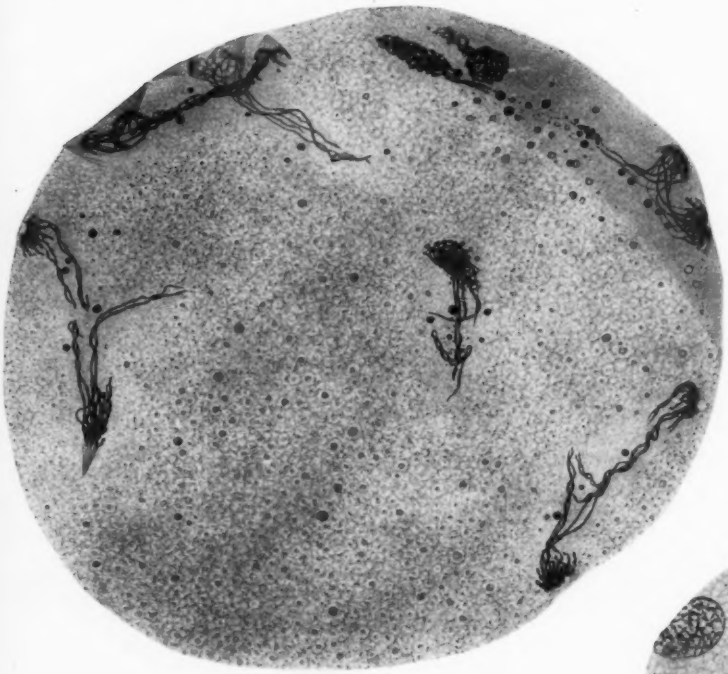

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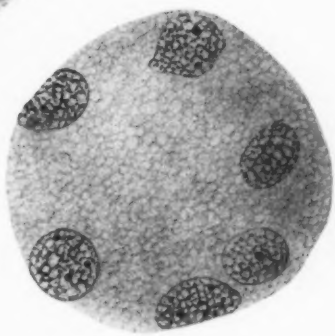

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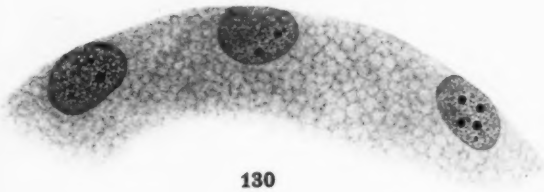

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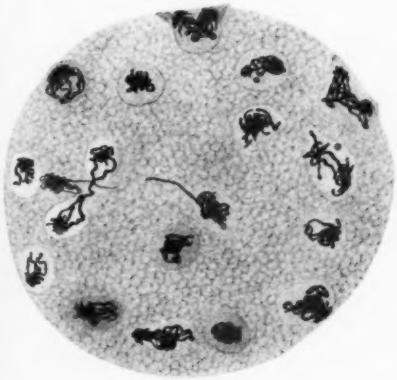

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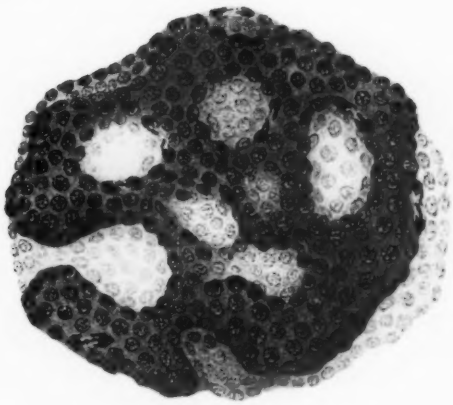

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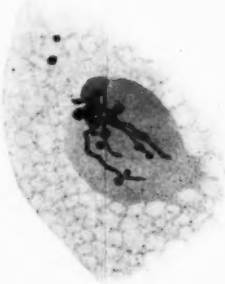

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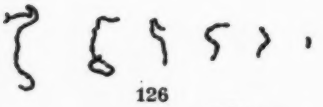

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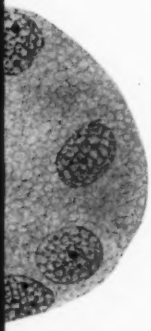

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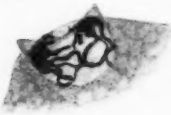

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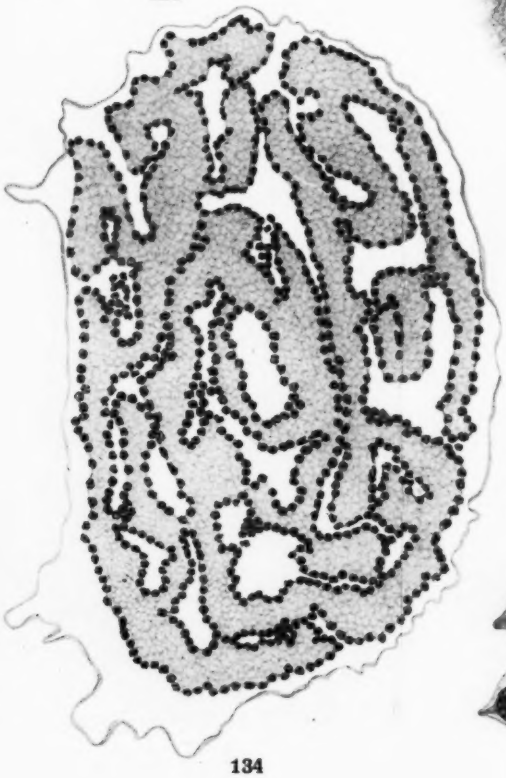

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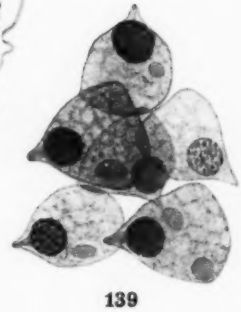

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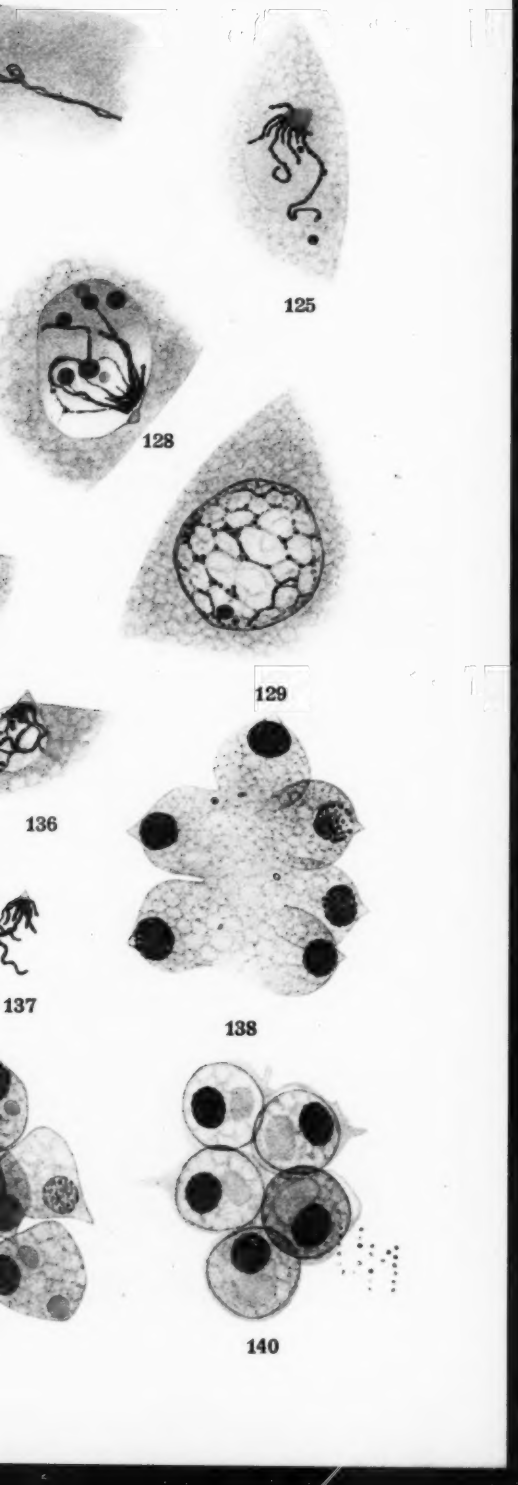

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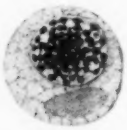
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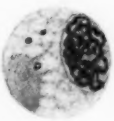
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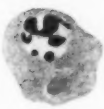
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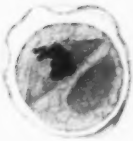
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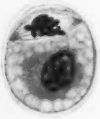
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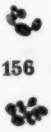
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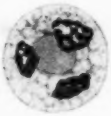
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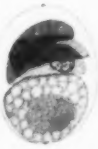
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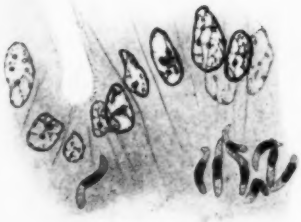
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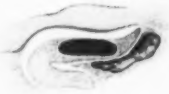
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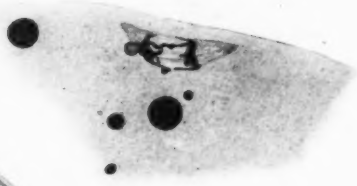
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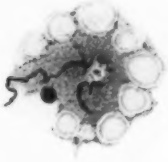
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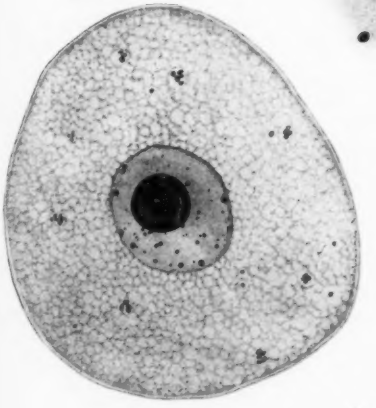
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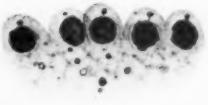
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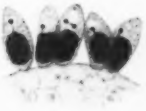
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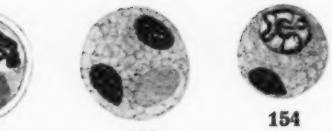
PLATE VI



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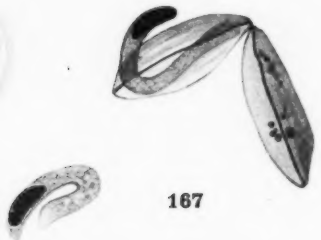

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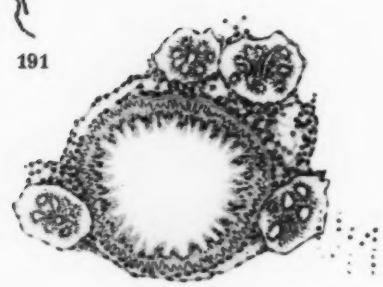

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